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Mestrado

Novel Ready-to-eat Mango Product using Gellan Gum as Gelling Agent:

Physico-chemical, Microbial and Sensory Characteristics

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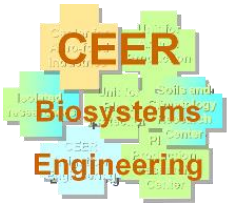

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To my mother,

Dedic această teză mamei mele,

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ABSTRACT

The development of novel food products designed to maximise both the health benefits and enjoyment of the consumer's eating experience is an important area of research nowadays. Fruits and vegetables are also increasingly recognized as important components of a healthy diet. Within this context, the work in this thesis aimed the development of a novel convenient food product – fresh like mango bars - based on mango (*Mangifera indica* L) and gellan gum, designed to have sensory quality similar to that of fresh mango, to be of easy consumption and to have an extended shelf-life.

The influence of gellan concentration and of the ratio of L (low acyl)/H (high acyl) gellan on the rheological and microstructural properties of mango/gellan gels was studied. This allowed optimising the concentration of gellan. The effect of the L/H ratio on texture, microstructure, syneresis and sensory acceptance was evaluated. Results separated bars into two sets: one showing a higher hardness and lower syneresis (only L gellan and L/H in the ratios of 75/25 and 50/50); and the other with bars having a higher proportion of H, with a softer structure with higher cohesiveness and syneresis (L/H25/75 and only H gellan). Through sensory analysis the bar with L/H 25/75 was identified as the preferred one.

An edible coating was developed to improve the characteristics of the product. Its optimization was performed by the use of experimental design and mathematical modelling. Results showed that the coating improved appearance and firmness of bars, and reduced syneresis and volatiles loss during storage.

Thermal and high hydrostatic pressure preservation treatments were applied on packaged products. Both treatments had similar performance considering microbial content and aroma release. Thermally treated bars were stored at 5 °C during 21 days, without changing significantly their texture, colour, microbial loading and sensory attributes.

Overall results show that the process allows producing a stable ready-to-eat mango bar product appreciated by the sensory panellists.

Keywords: mango fruit, convenient mango bars, gellan gum, rheology, coating, microstructure, preservation treatments,

RESUMO

O desenvolvimento de novos produtos alimentares, desenhados de forma a maximizar benefícios para a saúde e as características sensoriais, é uma área de investigação actualmente muito importante. As frutas e vegetais são cada vez mais considerados componentes essenciais numa dieta saudável. Neste contexto, o objectivo do presente trabalho foi o desenvolvimento de um produto alimentar pronto a consumir – barras de manga texturizada – baseadas em manga (*Mangifera indica* L) fresca e gelano que preservassem as propriedades sensoriais e funcionais da fruta fresca, facilitassem o consumo e apresentassem um tempo de prateleira adequado e superior ao da fruta.

Estudou-se a influência da concentração de gelano e da razão gelano L (baixo acilo)/H (alto acilo) nas propriedades reológicas e microestruturais dos géis de manga/gelano. Tal permitiu selectionar a concentração de gelano. Avaliou-se o efeito da razão L/H na textura, microestrutura, sinérese e aceitação sensorial. Barras produzidas com uma razão L/H > 50/50, apresentaram maior firmeza e menor sinérese; e com proporção mais elevada de H, menor firmeza e maior coesividade e sinérese. Testes de análise sensorial permitiram seleccionar a barra com L/H 25/75 para desenvolvimento posterior.

Foi optmizado um revestimento comestível (L/H 25/75) que implicou melhor aspeto e firmeza das barras, e reduziu a sinérese e a perda de compostos voláteis durante o armazenamento.

Barras embaladas em complexos laminados (polietileno e alumínio) foram submetidas a tratamentos de conservação térmicos e por altas pressões hidrostáticas. Os dois processos implicaram uma redução da carga microbiana e libertação de aromas equivalentes. Seleccionou-se o tratamento térmico (80 °C/120 s) para posteriores testes de conservação, tendo-se concluído que as barras mantiveram a textura, a cor, a carga microbiana e os atributos sensoriais quando armazenadas a 5ºC durante 21 dias.

Os resultados globais evidenciam que o processo permite produzir barras de manga prontas a consumir com uma estabilidade adequada e apreciadas sensorialmente.

Palavras chave: manga, barra conveniente de manga, gelano, reologia, revestimento comestível, microestrutura, tratamentos de conservação

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Nomenclature

Abbreviations

AAD	Absolute Average Deviation
AMB	Aerobic Mesophillic Bacteria
ANOVA	Analysis of Variance
BI	Browning Index
CCRD	Central Composite Rotatable Design
CLSM	Confocal Laser Scanning Microscopy
Cryo-SEM	Cryo-Scanning Electron Microscopy
FID	Flame Ionisation Detector
HHP	High Hydrostatic Pressure
H	High acyl gellan
L	Low acyl gellan
OAVs	Odour Activity Values
PCA	Principal Component Analysis
PE	Polyethylene
PET	Polyethylene terephthalate
RSM	Response Surface Methodology
SPME	Solid Phase Micro Extraction
SR	Stress Relaxation
TPA	Texture Profile Analysis
TSS	Total Soluble Solids
TT	Thermal Treatment
WHC	Water Holding Capacity

Variables

a*	Colour parameter (red-green) (dimensionless)
b*	Colour parameter (yellow-blue) (dimensionless)
b_0	Coefficient estimate for interception
b_i (i=1,2,3)	Coefficient estimate for linear correlation
b_{ij} (ij=1,2,3)	Coefficient estimate for quadratic correlation
E	Elastic modulus (Pa)
E_e	Equilibrium elastic modulus (Pa)

f	Frequency (Hz)
G'	Elastic (storage) moduli (Pa)
G"	Viscous (Loss) moduli (Pa)
k_1, k_2	Empirical Peleg's constants (dimensionless)
L*	Lightness (dimensionless)
T	Temperature (°C)
t	Compression holding time (s)
W	Weight (g)
X_i (i=1,2,3)	Experimental variables in RSM
Y_P	Predicted responses

Greek letters

α	Axial level in RSM
β	Exponent in the power law regression
ΔE*	Total colour difference (dimensionless)
λ	Relaxation time (s)
$\dot{\gamma}$	Shear-rate (s^{-1})
η	Viscosity (Pa s)
η_0	Zero shear-rate viscosity (Pa s)
η_a	Apparent viscosity (Pa s)
σ(t)	Stress at any time during the stress-relaxation test (Pa)
σ_0	Initial relaxation stress (Pa)

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Chapter 1

Introduction

1.1. Health benefits of fruits and vegetables consumption

Fruits and vegetables are major components of a healthy diet. Consumption of fruits and vegetables has been associated with many beneficial effects on consumers' health and is recommended by several organisations such as the World Health Organisation (WHO) and the Food Agriculture Organisation (FAO) (WHO/FAO 2003). WHO in 2013 estimated that approximately 1.7 million (2.8 %) deaths per annum worldwide could be related to low consumption of fruits and vegetables. The link between the consumption of specific food products and the risk of developing several diseases has been widely argued in the literature, demonstrating a protective role of a diet rich in fruits and vegetables. Daily consumption of fruits and vegetables may reduce the risk of occurrence of diseases, such as cardiovascular conditions, certain cancers, type II diabetes and obesity. The health promoting effect of fruits, vegetables is related with their bioactive phytochemicals constituents. More than 5000 individual phytochemicals have been identified, being phenolics, vitamins, minerals, dietary fibre and carotenoids the most important. Among all, phenolic, together with carotenoids, are the most studied phytochemicals (Liu, 2004). These phytochemicals act through a variety of mechanisms, such as reducing antioxidant stress, improving lipoprotein profile, lowering blood pressure, increasing insulin sensitivity, and improving hemostasis regulation (Dauchet et al., 2006).

The consumption of fruit and vegetables (8 servings (cups) /day) may improve the immune response, maintain eyesight, lower the incidence of certain types of cancer and heart disease, and prevent degenerative diseases. The results are equivalent to a 4 % reduction in risk for every 1 serving/day increase in the intake of fruits and vegetables (Joshipura et al., 2001).

According to the 'Instituto Nacional de Estatistica' the consumption of fruit in Portugal increased by 20.4 % in the period 1993 – 2003 (www.ine.pt). However, WHO in 2006 claimed that the consumption of fruit and vegetables in Portugal does not reach 400 g/day, which is equivalent with 5 servings (cups)/ day.

1.2. Consumer's perception about new products development

Lifestyles of modern consumers, along with the demand for natural, fresh, flavorful, convenient and high quality products with health benefits have raised the production and consumption of fresh like fruit products.

The market of processed fruits and vegetables, offers a variety of products such as sliced minimally processed fruits, juices and concentrates, pulps and dehydrated products, jams and jellies, pickles, chutneys and fruit bars (Ahvenainen, 1996; Schieber, Wieland, & Reinhold, 2000). Is this variety of processed fruits and vegetables enough for consumers' needs and perceptions?

In the numerous studies of new products performance over the years, there is the agreement that understanding consumer needs is of great strategic value, especially in the early stages of the new product development. To induce a change in consumer behaviour leading to an increase consumption

of fruit and vegetables, a wide variety of health and social marketing interventions have been conducted around the world for more than a decade. However, the success of these initiatives, measured in terms of increase in the daily consumption of fruit and vegetables per person remains modest (Rekhy & McConchie, 2014). The proposal of convenient food products, attractive to consumers, could be a way to increase consumption of fruits and vegetables, with a positive impact on consumer's health, as long as the processing methods do not modify the nutritional quality of the raw fruits or vegetables. For producers, the final product must have high yield and good appearance, must withstand long-distance shipping to markets. Appearance quality, firmness and shelf-life are important from the point of view of wholesale and retail marketers. Consumers judge quality of fruits products on the basis of appearance and firmness at the time of initial purchase (Kader, 1999). Consumers are also concerned about the nutritional quality of fruit products, which are not only colorful and flavorful components of our diet, but also a good source of energy, vitamins, minerals, dietary fibres and many bioactive compounds that bring a lot of benefits to the human health.

A conceptual approach that can promote an increase of the efficiency and effectiveness of the innovation processes was summarized by Urban and Hauser in 1993, explaining that the key stages in the formulation of the consumer-oriented new products development follows a closely market-oriented approach: 'need identification', 'idea development' to fulfil the need, 'product development' to substantiate the idea and the product's 'market introduction' communicating the fulfilment and need (Figure 1.1).

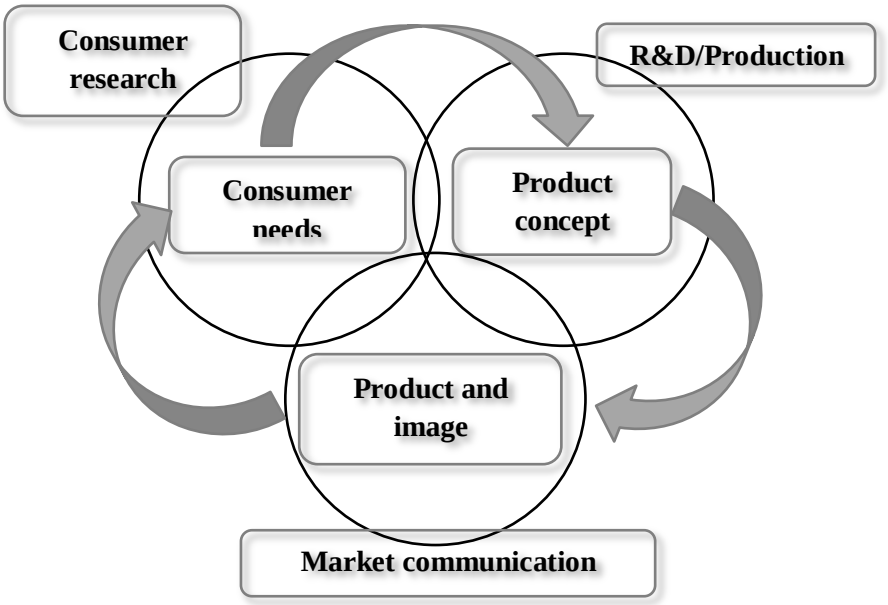

Figure 1.1 The consumer orientated new product development concept (Urban & Hauser, 1993).

The research of consumer's perception about the link between healthy life style and development of new products has often been used by both marketing and R&D. The development of new appealing

food products based on fruit and vegetables could be the key objective of food and nutrition policy interventions conducted around the world by government and non-government investors (Kader, 1999). Within this context, the work in this thesis aimed the development of a novel convenient food product – fresh like mango bars - based on mango (*Mangifera indica* L) and gellan gum.

1.3. Theoretical considerations

In this section, a literature review of the polysaccharides and of the most important techniques used is described.

1.3.1. Polysaccharides

Polysaccharides are very common and abundant in nature as, for example plant building material (cellulose) or reserve energy (starch), and as protective coatings for bacteria and other cells. They are long-chain carbohydrate molecules assembled by a large number of chemically-linked building blocks, so-called monomers, joined together by glycosidic bonds to form high molecular chains.

Nowadays, due to their biodegradability, biocompatibility, antimicrobial activity, non-toxicity, and versatile chemical and physical properties, polysaccharides are used in many areas of application, such as food industry, pharmaceutical and cosmetic industries (as thickeners, gelling agents, texture modifiers, stabilizers and biding agents), in the paper industry (as strengthening agents), in oil fracturing as well as coatings, in wastewater treatment and in biodegradable films, for agricultural purposes and packaging (Alves et al., 2010). The main advantages of polysaccharides are the relatively low cost, the simplicity of manufacturing complex shapes with vast range of functions in industrial sectors.

Polysaccharides can be divided into two main classes:

- *Homo-polysaccharide* which have only one type of monosaccharide repeating unit in the chain. Some homo-polysaccharides serve as storage forms of monosaccharaides that are used as fuels, (starch in plant cells and glycogen in animal cells). Other homo-polysaccharides serve as structural elements in plant cell wall and animal exoskeletons (cellulose and chitin).
- *Hetero-polysaccharide* which are composed of two or more types of monosaccharaides. They provide extracellular support for different organisms. For example, in animal tissues, the extracellular space is occupied by several types of hetero-polysaccharides which form a matrix that hold individual cell together and provides protection, shape and support to cells, tissues and organs (Lehninger, Nelson, & Cox, 2005). Another group of hetero-polysaccharides is represented by the primary cell wall of fruits and vegetables, including a wide variety of pectins and hemicelluloses as well as cellulose.

Various hetero-polysaccharides also called hydrocolloids or gums are, commercially available for use in food and non-food industries. Hydrocolloids of vegetable, animal, microbial or synthetic origin

are nowadays widely used in a variety of industrial sectors performing a number of functions including moister binding and control, structure and flow behaviour (Philips & Williams, 2000; Imeson, 2010).

Hydrocolloids have a significant impact on textural and organoleptic properties when used at levels ranging from parts per million to high levels. Due to their important role they continue to engage the attention to many expert researchers. Previous research in this area has concentrate mainly on the effect of food hydrocolloids on the perceived intensity of the four basic tastes (sweet, sour, bitter and salty) rather than on flavour (aroma) perception and has been usually restricted to investigation of differences between several gums over a small range of concentration of viscosities (Gormley & Holm, 2010).

Over recent years, many techniques have given more light on the fine structure of the hydrocolloids, to enhance the understanding of network formation and how they combine with other polymers (Imeson, 2010).

Microbial polysaccharides are polymers secreted by the cells to form a layer over the surface of the organism, either as a capsule that remains associated with the cell surface (e.g. K30 antigens) or as a slime that is loosely bound to the cell surface (e.g. xanthan, gellan, hyaluronic acid). Because of their position they are characterized as exopolysaccharides (EPS), to distinguish them from any polysaccharides that might be found within the cell (Morris & Harding, 2009). The functions are thought to be mainly protective, either as a general physical barrier preventing access of harmful substances, or more specific as a way of binding and neutralizing bacteriophage. In appropriate environments they may prevent dehydration (Kumar, Mody, & Jha, 2007).

Some bacterial EPSs can directly replace polysaccharides extracted from plants (e.g. guar gum or pectin) or algae (e.g. carrageenan or alginate) in traditional applications, because of their improved physical properties (e.g. xanthan gum or gellan gum) (Table 1.1), (Fialho et al., 2008; Rehm, 2009).

The global market for hydrocolloids, which includes many polysaccharides, is still dominated by plant and algal polysaccharides (e.g. starch, galactomannans, pectin, carrageenan and alginate). This market valued at > 4 million US$ in 2008, with xanthan gum being the only significant bacterial EPS, which accounted for 6% of the total market value (Imeson, 2010).

The polysaccharides referred in the previous paragraphs as well as most plant cell wall components are important elements of human nutrition, being known as nondigestible polysaccharides (NDP) and nondigestible oligosaccharides (NDO) or dietary fiber (Brownlee, 2011).

The physicochemical and biological properties of dietary fiber are associated with physiological actions in the small and large intestine, having important implications in human health. These properties include water dispersibility and solubility, viscosity effects, bulk, absorption and fermentability, and binding of other compounds. These features may lead to various physiological actions such as reducing cholesterol and attenuating blood glucose, maintaining gastrointestinal health, and positively affecting calcium bioavailability and immune function. Moreover, based on their

physiochemical properties, many of the new oligosaccharides can help to improve the organoleptic properties and nutritional value of foods (Tungland & Meyer, 2002).

Fruits and vegetables can interact with polysaccharides used in the food industry to form new textures and perform specific functions, therefore, many food scientists are now interested in the physiological effects of such polysaccharides in combination with fruit or vegetable.

Table 1.1 An overview of the most relevant physicochemical and functional properties, main areas of application and market assessment of some extensively studied bacterial EPS (Freitas et al., 2011)

EPS	Components	Change	Molecular weight	Main properties	Main applications	Market (metric tons)	Market value (US$)	Price (US$)/kg	Refs.
Xanthan	Glucose Mannose Glucuronic acid Acetate Pyruvate	Anionic	(2.0-50) x 10^6	Hydrocolloid -High viscosity yield at lower shear rates even at low concentrations -Stability over wide temperature, pH and salt concentrations ranges	-Food -Petroleum industry -Pharmaceuticals -Cosmetics and personal care products -Agriculture	96 000	235 millions	3-5	Rehm, 2009; Imenson, 2010; Ullrich, 2009; Yang, 2007;
Gellan	Glucose Rhamnose Glucuronic acid Acetate Glycerate	Anionic	5 x 10^5	Hydrocolloid - Stability over wide pH range -Gelling capacity -Thermoreversible gels	-Food -Pet food - Pharmaceuticals -Research: agar substitute and gel electrophoresis	Not available	15 millions	55-66	Rehm, 2009; Imenson, 2010; Fialho, 2008; Ullrich, 2009; Bajaj, 2007;
Alginate	Guluronic acid Mannuronic acid Acetate	Anionic	(0.3-1.3) x 10^6	Hydrocolloid Gelling capacity Film-forming	-Food hydrocolloid -Medicine	30 000	Not available	5-20	Rehm, 2009; Imenson, 2010; Pena, 2008 ;
Cellulose	Glucose	Neutral	~ 10^6	High crystallinity Insolubility in most solvents High tensile strength	-Foods (indigestible fiber) -Biomedical	Not available	Not available	5.8-12	Chawla, 2009 ; Rehm, 2009; Bae, 2004 ; Cheng, 2009
Dextran	Glucose	Neutral	10^6 - 10^9	Non-ionic Good Stability Newtonian fluid behaviour	-Foods Pharmaceutical industry Chromatographic media	2 000	Not available	N.A	Rehm, 2009; Rehm, 2010; Yang, 2007;

1.3.2. Rheology

Dynamic oscillatory shear measurements

Nowadays, structured fluids are extensively used. Most foods, cosmetics, and pharmaceuticals contain particles or droplets of an immiscible fluid suspended in a carrier liquid. A number of soft semisolid materials also fall under the category of structured fluids since they have a multiphase structure and exhibit complex flow behavior (Franck, TA Instruments).

Characterising the mechanical behaviour of soft materials is complicated by the fact that many materials are viscoelastic, so their mechanical properties lie between that of a pure elastic solid and that of a viscous liquid. Using dynamic oscillatory rheology, it is possible to quantify both the viscous-like and the elastic-like properties of a material at different time scales; being a valuable tool for understanding the structural and dynamic properties of these systems (Macosko, 1994; Larson, 1999).

The basic principle of an oscillatory rheometer is to induce a sinusoidal shear deformation in the sample and measure the resultant stress response; the time scale probed is determined by the frequency of oscillation, ω, of the shear deformation. In a typical experiment, the sample is placed between two plates, as shown in Figure 1.2. While the top plate remains stationary, a motor rotates the bottom plate, thereby imposing a time dependent strain $\gamma(t) = \gamma \cdot \sin(\omega t)$ on the sample. Simultaneously, the time dependent stress $\sigma(t)$ is quantified by measuring the torque that the sample imposes on the top plate (Lapasin, 1995).

Measuring this time dependent stress response at a single frequency immediately reveals key differences between materials, as shown schematically in Figure 1.3. If the material is an ideal elastic solid, then the sample stress is proportional to the strain deformation, and the proportionality constant is the shear modulus of the material. The stress is always exactly in phase with the applied sinusoidal strain deformation. In contrast, if the material is a purely viscous fluid, the stress in the sample is proportional to the rate of strain deformation, where the proportionality constant is the viscosity of the fluid.

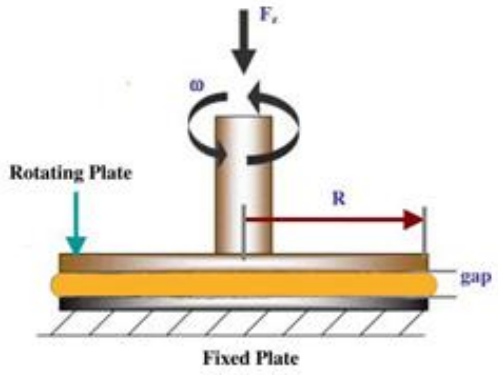

Figure 1.2 Schematic representation of a typical rheometry setup with the sample placed between the plates (Adapted from Wyss, Larsen, & Weitz, 2007).

Viscoelastic materials show a response that contains both in-phase and out-of-phase contributions, as shown in Figure 1.3. These contributions reveal the extents of solid-like and liquid-like behavior. As a consequence, the total stress response shows a phase shift δ with respect to the applied strain deformation that lies between that of solids and liquids, $0 < \delta < \pi/2$. The viscoelastic behaviour of the system at ω is characterised by the storage modulus, G' (ω), and the loss modulus, G"(ω), which respectively characterise the solid-like and fluid like contributions to the measured stress response. For a sinusoidal strain deformation $\gamma(t) = \gamma_0 \sin(\omega t)$, the stress response of a viscoelastic material is described by Equation 1.1 (Wyss, Larsen, & Weitz, 2007).

$$\sigma(t) = G'(\omega)\,\gamma_0 \sin(\omega t) + G''(\omega)\,\gamma_0 \cos(\omega t) \qquad \text{Eq. 1.1}$$

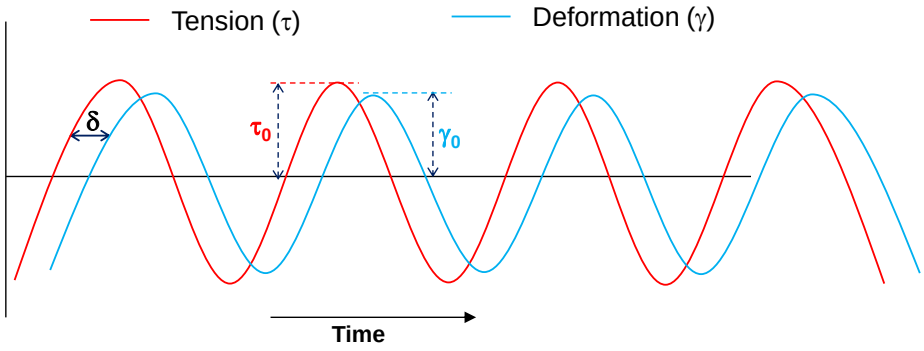

Figure 1.3 Schematic stress responses to oscillatory strain deformation for a viscoelastic material (Adapted from Wyss, Larsen, & Weitz, 2007).

Stress – relaxation

There are several mathematical models that can describe the behaviour of viscoelastic food products; nevertheless the Maxwell and Peleg models are more often used to describe the behaviour of the gels and food systems (Morales, Guerreiro, Serra, & Gou, 2007; Andrés, Zaritzky, & Califano, 2008; Bellido & Hatcher, 2009). The simplified Maxwell model involves two simple elements to represent different behaviours: an ideal elastic element represented as a spring and defined by an elastic constant (E), and an ideal viscous element, represented by a dashpot and having a behaviour defined by its viscosity (η) (Campus et al., 2010). The springs and dashpots can be connected in various ways to portray the behaviour of viscoelastic materials (Rodrígues-Sandoval, Fernandez-Quintero, & Cuvelier, 2009). This model is expressed by Equation 1.2.

$$\sigma(t) = \varepsilon_0\left(E.\exp\left(-\frac{t}{\lambda}\right) + E_e\right) \qquad \text{Eq. 1.2}$$

where σ (t) is the decreasing stress at any time during the relaxation test, ε_0 is the initial constant strain, E is the elastic modulus of the material, t is the compression holding time, λ is the relaxation time that is equivalent to by η/E and E_e is the equilibrium elastic modulus. Some food products do not follow the simplified Maxwell model, and thus, the description of their behaviour requires more complex models as the generalized Maxwell model. In this model several Maxwell elements are assembled in parallel (Figure 1.4) which the relaxation does not occur at a single time, but in a set of times. In this case, the stress-relaxation curves can be described by Equation 1.3:

$$\sigma(t) = \varepsilon_0 \left(E_1 \exp\left(-\frac{t}{\lambda_1}\right) + E_2 \exp\left(-\frac{t}{\lambda_2}\right) + \ldots + E_e \right) \quad \text{Eq. 1.3}$$

where E_i are the elastic moduli of the ideal elastic body; λ_i are the relaxation times.

The stress relaxation data can also be described using Peleg model (Kaletunc, Normand, Nussinovitch, & Peleg, 1991) (Eq. 1.4).

$$\sigma(t) = \sigma_e + \sigma_0 \left(\frac{k_1/k_2}{k_1 + k_2 . t} \right) \quad \text{Eq. 1.4}$$

where, σ_e is the residual stress, σ_0 is the initial relaxation stress, and k_1 and k_2 are empirical constants (Bhattacharya, 2010). The constant k_1 is a measure of how easy the material deforms. High values of k_1 suggest a hard material, which will dissipate less energy, and thus requiring more force to be compressed. The constant k_2 represents the degree of relaxation of the material.

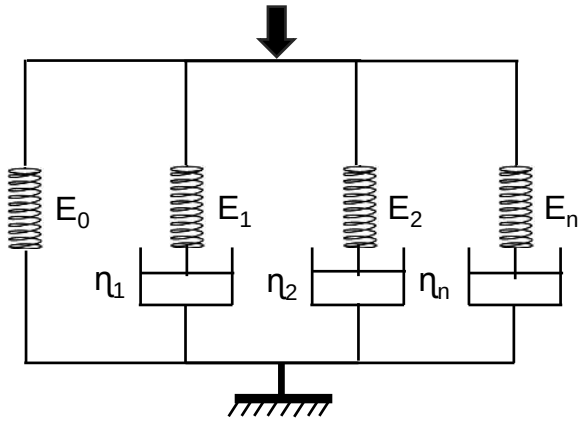

Figure 1.4 Representation of the generalized Maxwell model consisting on a single spring constant (E_0) in parallel to with Maxwell element models of dashpots (η_1 to η_n) accounting for the viscous behaviour and springs (E_1 to E_2) for the elastic behaviour (Adapted from Christensen, 2010).

1.3.3. Gas chromatography

Gas chromatography (GC) is the most widely used technique for the separation of volatile and semi-volatile organic compounds that can be vaporized without decomposition, in a mixture. Like for all other chromatographic techniques a mobile and a stationary phase are required (Jennings et al. 1997). The mobile phase (carrier gas) an inert gas i.e., helium, argon or nitrogen and the stationary phase is a microscopic layer of liquid or polymer on an inert solid support in a capillary column.

A sample port is necessary to introduce the sample at the head of the column. A calibrated microsyringe is used to deliver the sample volume in the range of a few microliters through a rubber septum and into the vaporization chamber (Mcnair & Miller, 2009). The vaporization chamber is heated above the lowest boiling point of the sample and subsequently mixed with the carrier gas to transport the sample into the column. The separation is based on different strengths of interaction of the compounds with the stationary phase. The stronger the interaction, the longer the compound interacts with the stationary phase, and consequently needs more time to migrate through the column (Li et al., 2009). The detector system is located at the end of the column which provides a quantitative measurement of the components of the mixture as they elute in combination with the carrier gas. Each detector has two main parts (the sensor and the electronic equipment) that when used together serve as transducers to convert the detected property changes into an electrical signal recorded as a chromatogram. The most generally applicable and widely used detector is flame ionisation detector (FID). In a FID, the sample is directed at an air-hydrogen flame after exiting the column. At the high temperature of the air-hydrogen flame, the sample undergoes pyrolysis (chemical decomposition) through intense heating leading to a release of ions and electrons that carry the current (Mendham et al., 2002).

Headspace-Solid Phase Micro Extraction

Headspace sampling is employed with gas chromatography (GC) in numerous fields and with a variety of applications (Snow & Slack, 2002). Headspace is the gas space above the sample in a vial. Volatile sample components diffuse into the gas phase, forming the headspace gas. Headspace analysis is therefore the analysis of the components present in that gas (Figure 1.5). Headspace gas chromatography is therefore a technique of gas extraction and can be comparable to a solvent extraction as a one-step extraction (static or equilibrium headspace) or as a continuous extraction (dynamic headspace) (Kolb, 1999).

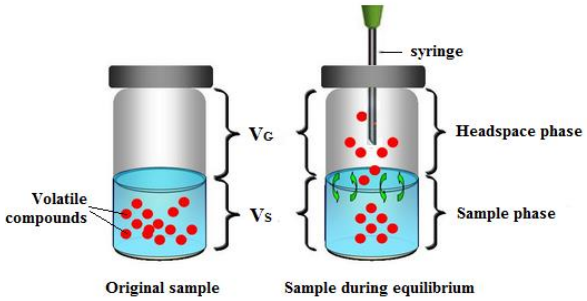

Figure 1.5 Headspace phases inside the vial before and during equilibrium (Adapted from Ciobanu et al., 2012).

Small concentrations of low-volatility compounds cannot be detected using only headspace technique even though they might contribute decisively to the olfactory perception of the aromas. To overcome this disadvantage, solid phase microextraction (SPME) together with gas chromatography may be used. SPME technique uses a fused silica fibre coated with an appropriate stationary phase as the extraction medium (e.g. polydimethylsiloxane) attached to a modified syringe (Stashenko & Martínez, 2007). SPME is essentially a two-step process. Firstly, the partitioning of analytes between the sample matrix, which can be a liquid sample or the headspace, and the desorption of the (concentrated) extract from the fibre into the analytical instrument (Figure 1.6) (Ibánez et al., 1998). This technique is used for small volumes of sample, maintaining and improving the sensitivity of the analysis (Stashenko & Martínez, 2007).

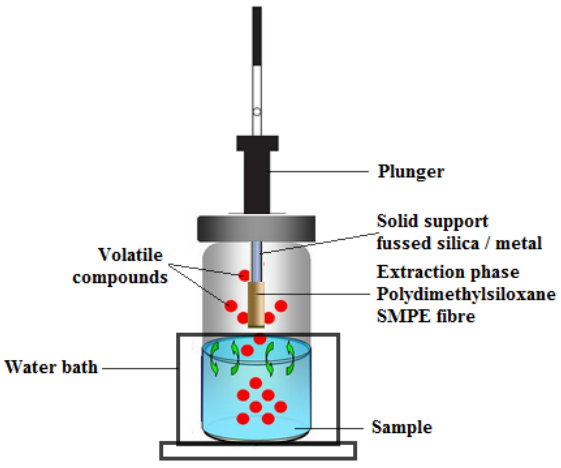

Figure 1.6 Illustration of the volatile compounds in equilibrium between three phases: sample, headspace and SPME fibre (Adapted from Chauhan et al., 2010).

1.3.4. High hydrostatic pressure (HHP)

High hydrostatic pressure, also known as ultra-high pressure (UHP), is an established non thermal food processing and preservation technique with reduced effects on nutritional and quality parameters compared to conventional thermal processing (Barbosa-Cánovas et al., 2005). At pressures higher than 300 MPa a significant inactivation of vegetative microbial forms is observed even at ambient temperatures within treatment times of several minutes. By increasing the pressure to 600 MPa (at present the technical limit of industrial scale equipment), most inactivation reactions are strongly accelerated (Toepfl, et al., 2006). Other benefits of this technology include the inactivation of some enzymes and gelation of proteins. It may also maintain nutritional and sensory attributes of food systems, and enable the development of products with novel characteristics (Barbosa-Cánovas et al., 2005).

HHP has been applied for many fruit and vegetable products such as strawberry puree and juice (Cao et al., 2012), pomegranate juice (Varela-Santos et al., 2012), guava puree (Yen & Lin, 1996), fruit purees (De Ancos, Gonzalez, & Pilar Cano, 2000) and blueberry juices (Barba et al., 2012).

Generally, there are two principles that govern the behaviour of foods under pressure: the Le Chatelier-Braun principle and the isostatic principle. The Le Chatelier-Braun principle indicates that any phenomenon (such as phase transition, change in molecular configuration, chemical reaction, etc.) accompanied by a decrease in volume is enhanced by the increase in applied pressure. The isostatic principle explains that the distribution of pressure into the sample is uniform and instantaneous. Thus, the process time is independent of sample size and shape (Ramaswamy et al., 2005).

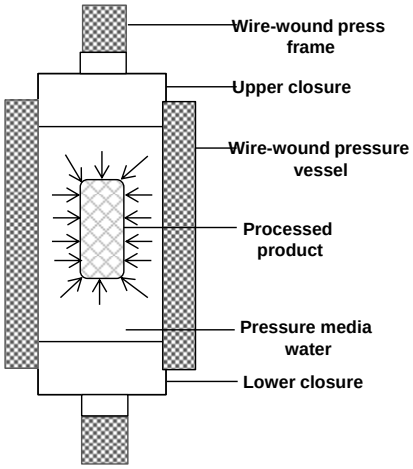

Figure 1.7 Schematic view of a high hydrostatic pressure equipment with wire-wound vessel and two-pressure transmission systems (Adapted from Barbosa-Cánovas et al., 2005).

The main part of the high-pressure equipment is the cylindrical pressure vessel, filled with a fluid, generally water, frequently built in alloy steel of high tensile strength. The wall thickness is designed to endure the maximum working pressure and the number of cycles for which the vessel is intended to.

Multilayers of wire-wound pre-stressed vessels are used for pressures higher than 600 MPa (Figure 1.7). These vessels are deliberately designed with a residual compressive stress to lower the maximum stress level on the vessel wall during pressurisation (Barbosa-Cánovas et al., 2005). The applied pressure is isostatically transmitted by the fluid (Pascal's law). In this way, a uniform pressure from every direction compresses the sample, which maintains the original shape. Pressure transmission is instantaneous, and it is independent of the product size and geometry. Contrary to heat, pressure transmission is non time/mass dependent, thus the time required for pressure to reach the internal points of the sample is minimized. However, pressure effects on microbial deactivation and on other food processing application are time dependent (Gould, 1995).

HHP has many advantages. An important advantage is that high pressure treatments at low processing temperatures have either no effect or a minimal effect on flavour, maintaining a maximum fresh-like flavor and taste of the food products. It can provide a safe product with reduced processing time. Moreover, it is environmentally friendly since it requires only electrical energy and no waste by-products generated (Ramaswamy et al., 2005, Toepfl et al., 2006). Due to these advantages, HHP has been widely used for food product preservation (Table 1.2).

Table 1.2

Examples of HHP effect on quality attributes of fruit beverages

Food products	*Treatment parameters*	*Storage conditions*	*Quality changes*	*References*
Blueberry juice	200 MPa, 15 min	Tested right after treatment	Total phenolic and anthocyanin content increased, whereas no changes in antioxidant capacity, pH, °Brix and Color	Barba et al., 2012
Blueberry juice	400-600 MPa 15 min	Tested right after treatment	Total phenolic and anthocyanin content increased; no changes in pH, °Brix and Color; but antioxidant activity decreased	Barba et al., 2012
Blood orange juice	400-600 MPa 15 min	4 °C for 10 days	93.4 % retention rate of anthocyanin; 85 % retention rate of ascorbic acid	Torres et al., 2011

1.4. Aim and scope of the thesis

One of the most important source of phytochemicals and a very popular world-wide tropical fruit is mango (*Mangifera indica* L.). Besides its attractive characteristics for consumers such as aroma, taste and texture, this fruit has in its composition molecules with biological antioxidant activities that can prevent several diseases (Alothman, Kaur, Fazilah, Bhat, & Karim, 2010; Liu, 2003).

Maturity at harvest is the most important factor that determines storage-life and final fruit quality. Despite their phytochemical source potential it is known that mango is a highly perishable climacteric fruit. Its firmness decreases quickly with high susceptibility to bruising during shipping and storage (Mitcham and McDonald, 1992). Pulp firmness change is an important quality factor that affects not only consumer acceptance but also susceptibility to bruising and compression resistance during transportation and handling.

Changes in mango fruits firmness appear as tissue softening and loss of cohesiveness. These results from modifications of the mango fruit cell walls are frequently attributed to the enzymatic degradation of cell wall materials (Gonzales-Aguilar, et al., 2008). Several studies have been done in order to understand the conditions where the textural changes occurred, but the effects of ripening and storage on the changes of cell walls of mango fruit are one of the most difficult issues to control. The development of new mango based products could contribute to increase the shelf-life of over ripen mangos and to obtain more convenient forms of consumptions and, simultaneously, to facilitate the change in consumers behaviour increasing the consumption of fruits.

In view of this, the main goal of this thesis is the development of a novel convenient mango product in the form of a bar with similar sensory quality attributes of a fresh mango fruit. The thesis rational is presented schematically in Figure 1.8.

This study also aimed understanding the effect of gellan gum, the hydrocolloid used on the overall quality of the developed mango bars. This polysaccharide was selected due to its ability to produce a wide range of gel structures with designed textures, and due to its good flavor release (Gibson & Sanderson, 1997; Bayarri et al., 2007).

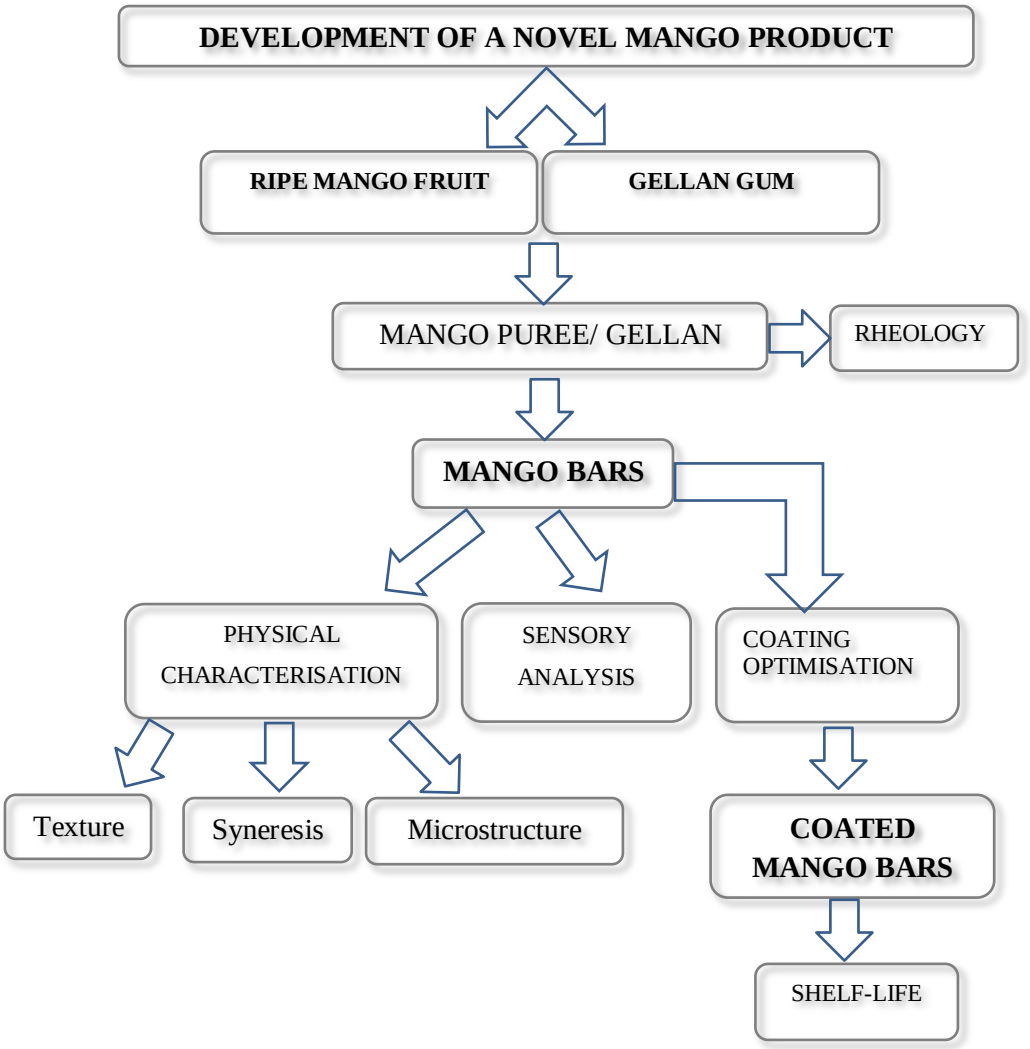

Figure 1.8 Thesis rational.

1.5. Thesis outline

This thesis is organised into six chapters, describing the work performed during this PhD project and its aims. The methodology used in each individual chapter is detailed in the context of the respective subject and, when applicable, is related to the one used in the previous chapters.

Chapter 1 introduces the subject of this thesis by presenting theoretical considerations, aim and scope of this PhD work.

Chapter 2 presents the influence of gellan concentration and of the ratio of low acyl/high acyl gellan on the rheological and microstructural properties of mango puree/gellan gels. Small amplitude oscillatory shear measurements were applied to evaluate the gel setting temperature, the stability of the

gel structure over time and the mechanical spectra of mango puree/gellan mixtures. The structural organisation of the mango puree and mango bars was visualized by Confocal Laser Scanning Microscopy (CLSM).

Chapter 3 describes the influence of the low/high acyl gellan ratio on the properties of mango bars, such as syneresis (passive diffusion and centrifugal dehydration), texture properties (Texture Profile Analysis and Stress Relaxation tests), microstructure (Cryo-Scanning Electron Microscopy) and texture sensory acceptance (Preference-Ranking test).

Chapter 4 is focused on the optimisation of an edible coating to improve the characteristics of the developed ready-to-eat mango bars. A central composite rotatable design (CCRD) was performed to optimize the formulations the coating, which was based on a mixture of low acyl/high acyl gellan as gelling agent.

Chapter 5 presents the results of the effect of two conservation treatments applied on packaged mango bars (thermal treatment and high hydrostatic pressure) on the microbial content and aroma release. The study of the characteristics of thermally treated packaged mango bars stored during 21 days under refrigerated conditions was carried out, in which the texture, colour, microbial loading and sensory attributes, were monitored.

Chapter 6 presents the conclusions and provides suggestions for future research.

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Chapter 2

Rheological and microstructural studies of mango puree/gellan gum mixtures

2.1. Summary

This work aimed the development of mango bars, made with fresh mango puree and gellan gum. The influence of gellan concentration as well as the ratio of L (low-acyl) and H (high-acyl) gellan on the rheological and microstructural properties of the mango bars was studied. Rheological outcomes showed that both L and H, and in mixtures, were able to produce jellified products, within a rather low maturation time. Furthermore, a synergistic effect was observed at different ratios of L/H, enabling the production of mango bars with intermediate viscoelastic properties. Higher values of the dynamic moduli were perceived as the L content increased. Confocal microscopy is in agreement with the rheological results, revealing a weakening of the gel structure with the increasing percentage of H. Results suggest that 1.0 %wt of L/H 50/50 produce structures with suitable mechanical properties. However, the results need to be correlated to texture and sensory analysis, before proceeding to the next steps of product development.

2.2. Introduction

Changes in modern lifestyle and the growing awareness of the link between diet and health, as well as new processing technologies, have led to a rapid rise in the consumption of fresh fruits products (O'Shea, Arendt, & Gallagher, 2012). Ready-to-eat fresh fruit products became an important area in the food industry due to their characteristics of freshness, low caloric content, and an active promotion of fruit as a basic component of a healthy diet (Corbo, Lanciotti, Gardini, Sinigaglia, & Guerzoni, 2000). Therefore, new trends in food industry require the development of new high quality convenient fruit products compatible with a healthy diet. However, it is difficult to obtain a convenient product maintaining the desired stability from a chemical, enzymatic and microbiological point of view and presenting the nutritional and sensory attributes of a fresh product. A key factor on the development of new products relies on the selection of the type of fruits to be used, taking into account their nutritional value, physico-chemical and sensory properties and the characteristics of the desired final product.

Mango *(Mangifera indica* L.) is a popular and economically important tropical fruit throughout the world, due to its good texture, flavour. The consumption of mango can provide significant amounts of important nutrients with high content of carotenoids, vitamin C, vitamin E, phenolic compounds, minerals and fibre, (Table 2.1) (Charles, Vidal, Olive, Filgueiras, & Sallanon, 2013; Vijaya, Sreeramulu, & Raghunath, 2010; Xiaolin, Guoxing, Yan, Tianjia, Yueming, & Jianrong, 2011). Its daily intake in the diet has been related to prevention of degenerative processes such as cardiovascular diseases, type II diabetes and obesity (Alothman, Kaur, Fazilah, Bhat, & Karim, 2010; Liu, 2003; Sánchez-Robles, Rojas-Graü, Serrano-Odriozola, Gonsalez-Aguilar, & Martín-Belloso, 2009).

Mango has been processed into a large variety of products, such as juices, powders, purees and dehydrated slices. Furthermore, among the derivative mango products, mango bars or mango leathers are the most popular fruit bars in India. This mango bar is traditionally prepared by adding cane sugar, spreading the puree on bamboo mats and, drying it in the sun into leathery sheets. Mango gels were developed using processed commercial mango juice and sodium alginate, with the addition of a calcium source (calcium orthophosphate) and a calcium sequestrant (glucono-δ-lactone) (Vijayanand et al., 2000; Dak, Verma, & Jaaffrey, 2007; Sriwimon & Boonsupthip, 2011; Djantou et al., 2011; Ledeker et al., 2014; Sogi, Siddiq, & Dolan, 2014; Roopa & Bhattacharya, 2014).

Table 2.1 The main components present in the fresh mango fruit. Nutrition value per 100 g.

Principle	Nutrient value	Percentage of RDA
Energy	70 kcal	3.5 %
Carbohydrates	17 g	13 %
Protein	0.5 g	1 %
Total fat	0.27 g	1 %
Dietary fibre	1.8 g	4.5 %
Vitamins		
Folates	14 µg	3.5 %
Niacin	0.584 mg	3.5 %
Pantothenic acid	0.160 mg	1 %
Pyridoxine (Vit B6)	0.134 mg	10 %
Riboflavin	0.057 mg	4 %
Thiamine	0.058 mg	5 %
Vitamin C	27.7 mg	46 %
Vitamin A	765 IU	25.5 %
Vitamin E	1.12 mg	7.5 %
Vitamin K	4.2 µg	3.5 %
Electrolytes		
Sodium	2 mg	0 %
Potassium	156 mg	3 %
Minerals		
Calcium	10 mg	1 %
Copper	0.110 mg	12 %
Iron	0.13 mg	1.5 %
Magnesium	9 mg	2 %
Manganese	0.027 mg	1 %
Zinc	0.04 mg	0 %
Phyto-nutrients		
α - carotene	17 µg	-
β - carotene	445 µg	-
Crypto-xanthin-β	11 µg	-
Lutein-zeaxanthin	0 µg	-
Lycopene	0 µg	-

RDA – Recommended daily allowance. (Source: USDA National Nutrient data base).

Gellan gum, a polysaccharide approved as a food additive has been used in a wide variety of food products including confectionary, jams, jellies, fabricated foods, hydrogels, pie fillings, puddings, ice cream and yogurt (Tang, Marvin, Tung, & Zeng, 1996; Lau, Tang, & Paulson, 2000; Noda et al., 2008). This hydrocolloid was chosen as gelling agent for the fresh mango bars due to its ability to produce a wide range of gel structure and good flavor release (Bayarri, et al., 2007). The native polymer, high acyl (H) gellan, is an extracellular hydrocolloid secreted by the bacterium *Pseudomonas elodea*, and its linear structure is based on the tetrasaccharide repeating unit →4)-L-rhamnopyranosyl-(α-1→3)-D-glucopyranosyl-(β-1→4)-D-glucoronopyranosyl-(β-1→4)-D-glucopyranosyl-(β-1→ with O (2) L – glyceryl and O (6) acetyl substituents on the 3-linked glucose (Yamamoto & Cunha, 2007;

Noda et al., 2008; García, Alfaro, Calero, & Muňoz, 2011). When exposed to alkali at high temperature, the acyl groups are hydrolysed and the deacylated form, low acyl (L) gellan, is obtained (Figure.2.1).

H gellan hydration is inhibited by the presence of sugars and high pH values; therefore, the hydration must be carried out in presence of less than 40 °Brix total soluble solids (TSS) content and at pH values below 4. By the contrary, L gellan hydrates easily in the presence of sugars (up to 80 °Brix TSS), being its hydration much more dependent on the ions concentration, and generally it is not complete at pH values below 4 (Philips & Williams, 2009).

The gelation process of both gellan types is generally considered to involve two separate steps. In aqueous solutions, upon heating, the molecules of gellan are in a disordered coil state (single chain). Upon cooling the molecules adopt an ordered double helical conformation followed by associations between the helices through weak interactions such as hydrogen bonds and van der Waals forces (Nickerson, Paulson, & Speers, 2003; Matsukawa & Watanabe, 2007; Picone & Cunha, 2010). In the case of L gellan, the helices aggregation are also promoted by pH reduction or mediated by cations, either by site binding between pairs of carboxylate groups on neighbouring helices or by suppressing electrostatic repulsion by binding to individual helices (Morris, Nishinari, & Rinaudo, 2012).

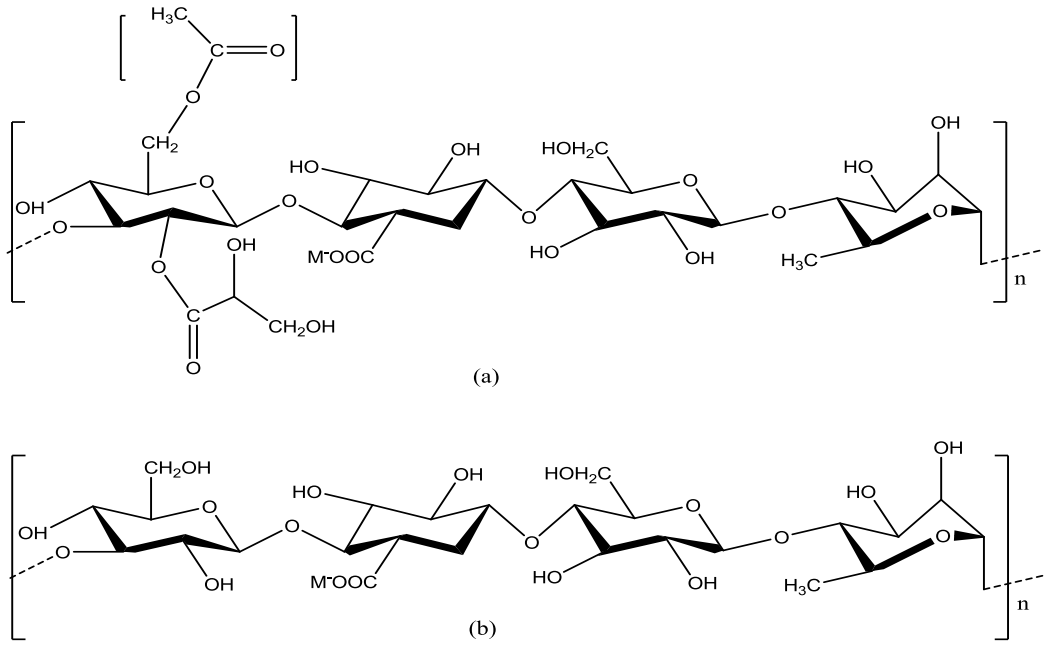

Figure 2.1 Chemical structure of gellan gum repeat unit: (a) native form H (High acyl gellan), (b) deacylated form L (Low acyl gellan).

Generally, H gels are thermally reversible without thermal hysteresis and set at temperatures around 70-80 ºC; while the gel setting temperature of L gels is around 30-50 ºC showing significant

thermal hysteresis, which extent is dependent on the nature and concentration of cations in solution (Philips & Williams, 2000; Rocha, Gonçalves, Bicho, Martins, & Silva, 2014).

One of the major advantages of gellan gum relies on the ability of L and H to produce gels with quite different characteristics. H gellan usually produces elastic, soft, non-brittle and opaque gels and L gellan enables the formation of non-elastic, hard, brittle and transparent gels (Philips & Williams, 2009; Ogawa, Takahashi, Yajinma, & Nishinari, 2006). Therefore, a wide range of structures, with varied rheological properties, appealing textures and good flavour release, may be designed by controlling the acyl content.

The development of texturized fruit bars involves the understanding of synergism between gellan gum and mango puree. The final structure and textural properties of these systems are strongly dependent on gellan physico-chemical properties (e.g. acylation degree), pH and composition of the puree (e.g. sugars, cations and fibre content), as well as on processing conditions such as gellan concentration, L/H ratio and temperature.

The influence of the addition of gellan gum (different concentrations and L/H ratios) to the mango puree was evaluated through analysis of the rheological (small amplitude oscillatory shear measurements) and microstructural (confocal laser scanning microscopy) properties in order to develop a new fresh mango bar.

2.3. Materials and methods

2.3.1. Materials

Two types of commercial food grade gellan gum: low-acyl gellan (Kelcogel® F) and high-acyl gellan (Kelcogel® LT) were supplied by CP Kelco Corporation, Wilmington, USA: The composition of gellan gum powder was supplied by the manufacturer. L gellan: carbohydrate (dietary fibre) 82 g/100 g; moisture 7 g/100 g; Ca^{2+} 252 mg/100 g; P 115 mg/100 g; Fe^{2+} 4 mg/100 g; Mg^{2+} 91 mg/100 g, Na^{+} 478 mg/100 g and K^{+} 4650 mg/100 g. H gellan: carbohydrate (dietary fibre) 78 g/100 g; moisture 6 g/100 g; Ca^{2+} 259 mg/100 g; P 233 mg/100 g; Fe^{2+} 2 mg/100 g; Mg^{2+} 98 mg/100 g, Na^{+} 510 mg/100 g and K^{+} 1930 mg/100 g.

Mature mangoes (*M. indica* L. cv. Palmer) were purchased from a local supermarket, Lisbon, Portugal and stored at 5±1 ºC until processing (within 24 h). Fruits were selected based on the same ripening stage (soft-ripe - best stage for consuming), uniform size and absence of any physical damage. Only one mango cultivar was selected because chemical composition may vary with the type of cultivar, and consequently, change the properties of the mango bars.

2.3.2. Methods

Preparation and analysis of fresh mango puree (control)

Mangos were stored at 5±1 ºC until processing (within 24 h). The fruit was then washed under running water, and manually peeled with a knife, cut into small pieces, and pureed in a food blender Vorwerk Thermomix TM-31 at 134 x g for 4 minutes at 22±2 ºC.

Determination of ash content

The total ash content was determined according to AOAC Official Method 923.03 (AOAC, 1990). A mass of 5 g of mango puree was weighed into a shallow dish and subjected to a temperature of 550 ºC until light a grey ash was produced. The dish was then cooled in a desiccator and weighed soon after reaching room temperature. The total ash content was calculated using Equation 2.1.

$$\text{Total ash} = \frac{\text{ash weight}}{\text{original sample weight}} \times 100 \qquad \text{Eq. 2.1}$$

Determination of mineral content

The ash obtained according the method described previously, was dissolved in 3 mL concentrated nitric acid and then diluted to 25 mL with deionized water. The solution obtained was used to determine the mineral content using an atomic absorption spectrophotometer (UNICAM M series, UK). Six types of minerals were determined, potassium, calcium, magnesium, sodium, and iron. Phosphorus was assayed colorimetrically based on the reaction of phosphate with molybdovanadate complex (Windham, 1995). The minerals content was expressed in mg/100 g edible portion. The analysis was carried out in triplicate

Determination of moisture content

A mass of 3 g of the mango puree (w_1) was placed in the oven for 6 h at 105 °C (in triplicate). After drying, the dried sample was weighed (w_2), and the moisture content was calculated using the following equation:

$$\text{Moisture content (\%)} = \frac{w_1 - w_2}{w_1} \text{ x } 100 \qquad \text{Eq. 2.2}$$

Evaluation of pH and Total Soluble Solids

The pH was determined using a pH meter BASIC 2º CRISON, Spain, calibrated with standard buffers at 6.89 and 4.01. A digital refractometer PAL-1, USA, calibrated with distilled water was used to determine Total Soluble Solids (TSS). Each analysis was carried out in triplicate.

2.3.3. Rheological measurements

To perform the rheological measurements, sample preparation consisted on dispersing the required gellan powder into 20 g of puree, under stirring for 10 min on a magnetic plate stirrer at room temperature (22±2 ºC). Then, the sample was transferred to the rheometer plate where the jellification, gels maturation and mechanical properties determination took place.

To study the effect of gellan concentration on the rheological properties of texturized mango puree, samples were prepared without gellan addition (control) and with 0.25, 0.5, 1.0 and 2.0 %wt of polymer, either using L or H gellan. To evaluate the effect of the L/H ratio on the rheological properties of the mango puree, samples were prepared in the same way without gellan (control) and with 1.0 %wt polymer at L/H ratios : 100/0 (L), 75/25, 50/50, 25/75 and 0/100 (H). The influence of 0.25, 0.5, 1.0 and 2.0 %wt of L/H 50/50 on the rheological properties of texturized mango puree was also analysed.

Small amplitude oscillatory shear measurements were performed with a controlled Stress Rheometer, RS 75 Rheostress HAAKE, Germany, connected to a thermostatic bath for the temperature control. The samples were placed between two parallel stainless steel serrated plates with a diameter of 35 mm, maintaining a constant gap of 1.0 mm. Liquid paraffin was applied along the border of the plates to prevent sample dehydration.

To evaluate the gel setting temperature, the experimental procedure consisted on subjecting the samples to 90 ºC for 1 min, followed by temperature sweep of oscillatory measurement from 90 ºC to 20 ºC at a rate of 1 ºC/min, f = 0.1 Hz and τ = 1.0 Pa. In the case of H gellan, the temperature range was between 95°C and 20°C in order to detect the gelling temperature. Immediately after the temperature sweep, a time sweep (maturation) was applied to investigate the change and the stability of the gels structure as a function of time. The maturation of the gels was monitored at 5 °C during 2 hours at constant stress (τ = 1.0 Pa) and frequency (f = 0.1 Hz*)*.

The samples were tested over a range of shear stresses. The stress sweep was applied under oscillatory shear of f = 0.1 Hz at 5 °C within a stress range between $\tau = 10^{-1}$ and 10^{4} *Pa*. Afterwards, to obtain the mechanical spectra, a new proportion of the same formulations were poured into the rheometer plate, and subjected to the temperature and time sweep tests described previously with the same oscillatory conditions. Then, frequency sweeps were conducted at 5 °C, for frequency ranging from 10^{-2} to 10^{2} *Hz*, with a constant shear stress within the linear viscoelastic region.

The frequency dependency was quantified from the β exponent values obtained by fitting the power law regression $G'(G'') = \alpha f^{\beta}$ (Valdez, Acedo-Carrilo, Rosas-Durazo, Lizardi, & Goycoolea, 2006). Each experiment was carried out in triplicate and reproducible results were obtained.

2.3.4. Confocal Laser Scanning Microscopy Analysis

For confocal laser scanning microscopy analysis, mango bars were also prepared without gellan (control) and with 1.0 %wt polymer at L/H ratios: 100/0 (L), 75/25, 50/50, 25/75 and 0/100 (H).

The procedure consisted on transferring 80 g of the puree obtained into a 100 mL beaker, immersed in a boiling water bath (to prevent heat burn of the puree) on a heater/magnetic stirrer, and homogenizing it by stirring at 134 x g. When the puree reached a temperature of 88±2 °C, the required gellan gum was added, followed by increasing the rotation speed up to 1640 x g for 30 seconds in order to have a good dispersion and dissolution of the gellan powder. Afterwards, for the non-covalent labelling, fluorescent probe Fluoresceine (0.1 g fluorescein/500 g) was added under stirring. The sample was then placed into rectangular silicon moulds (W x H x L = 50 x 10 x 25 mm) and allowed to set at room temperature (22 ± 2 °C). Afterwards the samples were stored at 5 °C for another 30 minutes.

Confocal Laser Scanning Microscopy (CLSM) of mango puree and thin slices of mango fruit bars with different L/H ratio was performed with a Leica TCS SP5, (Leica Microsystems, Mannheim, Germany) inverted confocal microscope using a 63 X apochromatic water immersion objective lenses with a NA of 1.2 and an Argon laser for excitation. Fluorescence was analysed using a 488 nm excitation wave length and emission was collected between 510 and 670 nm. The fluorescent probe, Fluorescein standard (Fluka, USA) was used for the non-covalent labelling of the mango puree and mango puree/gellan gum mixtures. The autofluorescence of mango puree was detectable in the same conditions. Microscopic observations were made at 20 °C and digital image files were acquired with 2048 x 2048 pixels for a typical sample area of 246x246 µm.

2.4. Results and discussion

2.4.1. Composition of mango puree

Among the minerals identified in mango puree, potassium was the one at higher concentration: 157.6±2.3 mg/100 g. The concentration of the other minerals was: calcium 12.5±1.6 mg/100 g; magnesium 9.4±0.8 mg/100 g; phosphorus 8.4±2.3 mg/100 g; sodium 2.2±1.8 mg/100 g and iron 2.6±0.6 mg/100 g. The ash content was 300±25 mg/100 g. The cation content of the mango puree and of both L and H gellan gum was enough to promote the gelation, as can be seen in the following sections. As such, there was no addition of external cations to the gellan/mango puree mixtures.

The water content was high (81 ± 0.03 %), therefore, the hydration of gellan was promoted without the addition of water.

The TSS content of the mango puree used was 18.0±0.5 ºBrix whereas the pH was 4.3±0.3. These values are in line with ideal conditions for the hydration of both L and H gellan: minimum pH value of 4 for L gellan and TSS content below 40 °Brix for H gellan (Philips & Williams, 2009).

2.4.2. Viscoelastic properties of the control and mango puree/gellan systems

This section is focused on the kinetics of gel network formation bearing in mind the different physico-chemical characteristics of the two types of gellan referred in the literature (Mao, Tang, & Swanson, 2000; Huang, Tang, Swanson, & Rasco, 2003).

Effect of concentration and type of gellan on the gelation kinetics and viscoelastic properties of texturized mango puree

The effect of type of gellan and its concentrations on the variation of the storage (G') and loss (G") moduli with temperature of mango puree/gellan mixtures is shown in Figure 2.2.

Temperature sweeps indicated that all evaluated samples exhibited G' larger than G" in whole temperature range, with no crossover between the moduli. This fact happens even at high temperatures where gellan molecules are still in a random coil conformation and the sample structure is mostly dependent on the mango puree itself. The initial gelation temperature was defined as the one at which an increase of both moduli is observed, around 45 ± 1.2 °C for 0.25 %wt L gellan and 64 ± 1.0 °C for 2.0 %wt L gellan. G' and G" continued to increase with decreasing temperature achieving a maximum value equivalent to the final gelation temperature around 37.0 ± 1.3 °C for 0.25 %wt L gellan and 50 ± 1.2 °C for 2.0 %wt L gellan. The initial and the final gelation temperatures became higher as the gellan concentration increased. In fact, the increase in polymer concentration offers more facility to establish intermolecular interactions, which is also in correlation with the increase of the dynamic moduli. For all gellan concentrations, the values of the moduli tend to a plateau after reaching a maximum value; this may be attributed to the beginning of the formation of a fully developed internal network. It is interesting to note that a decrease of G' and G" for temperatures below 50 °C for mango puree in the presence of 2.0 %wt L gellan was observed (Figure 2.2). This can be justified by the presence of fracture within the sample or slippage between the sample and the measuring geometry. Richardson & Goycoolea (1994) observed similar a behaviour with deacylated konjac glucomannan where the values of G' and G" decreased during gelation. Further experiments are needed in order to confirm the presence of fracture and/or slippage.

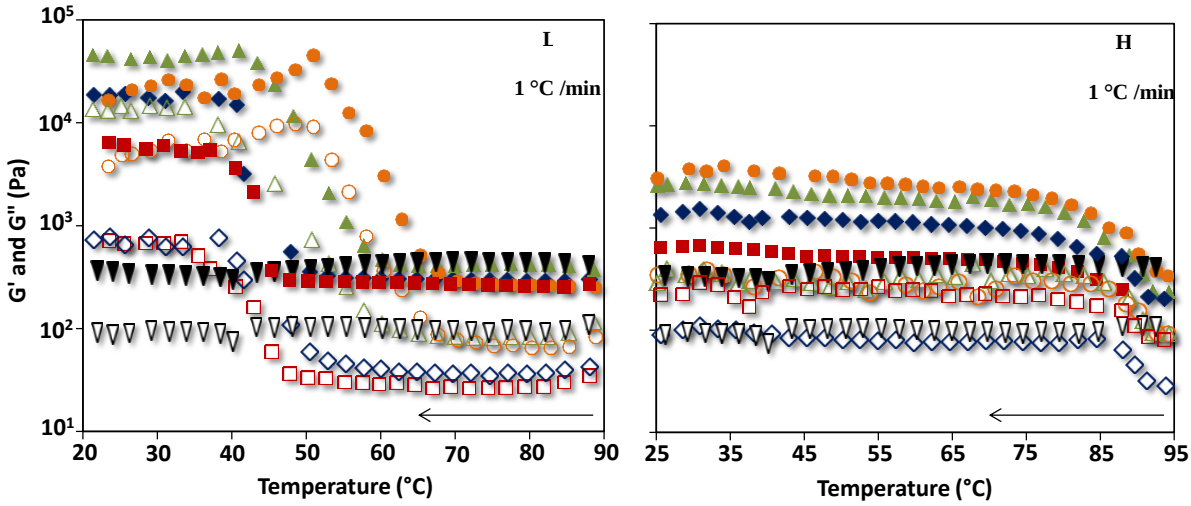

Figure 2.2 Cooling curve of mango puree with different L and H gellan concentration, full symbols – storage modulus (G'), open symbols – loss modulus (G''); (▼) control, (■) 0.25 %, (♦) 0.5 %, (▲) 1.0 %, (●) 2.0 %.

The pectin molecules present in the mango puree are expected to play an important role on the gel formation kinetics and on the final gel characteristics. This may happen because both L gellan and high methoxyl pectin form gels which properties are influenced by the presence of cations (e.g. calcium) (Miyoshi & Nishinari, 1999; Basu & Sivhare, 2010). Cations may suppress the electrostatic repulsions between the negative charge of pectin and gellan respectively, promoting gelation, and eventually the formation of a denser polymer network. However, further studies strictly focused on the interactions between both gellan types and pectin molecules in the presence of calcium are needed, to fully comprehend how the tridimensional carbohydrate network is formed within the complex system that is the mango puree.

When H gellan was used as gelling agent, a slight increase of the moduli was noticed around 90 °C corresponding to the gelation temperature. A well-defined plateau is perceived for temperatures below 65 ºC for all H gellan concentrations tested, with the values of the moduli becoming higher as the polymer content increased (Figure 2.2). For mango puree/H systems, more acyl groups lead to a greater inter-chain repulsion, therefore, less dense network formation with reduced vales of the moduli was noticed (Basu & Sivhare, 2010).

Immediately after the temperature sweep, samples were cooled down to 5 °C at 1°C/min and the gel maturation kinetics of the samples were monitored through the evolution with time of G' and G'' moduli (Figure 2.3). All the formulations with gellan gum reached a steady-state revealing constant values of both moduli. This information indicates that the gels formed have a stable and fully developed structure in a rather short period of maturation (30 minutes). This fact is quite important, because it may increase significantly the fruit bars production rate at an industrial scale. The structure of the mango puree (control) changes with time, the dynamic moduli shows a tendency to decrease

after almost 90 minutes of maturation. This may be attributed to the thixotropic properties of mango puree where the increasing degree of structural breakdown with time is due to shearing (Bhattacharya, 1999). For this type of soft solids, rheological properties change not only with shear stress but also with time of shearing (Tiu & Boger, 1974).

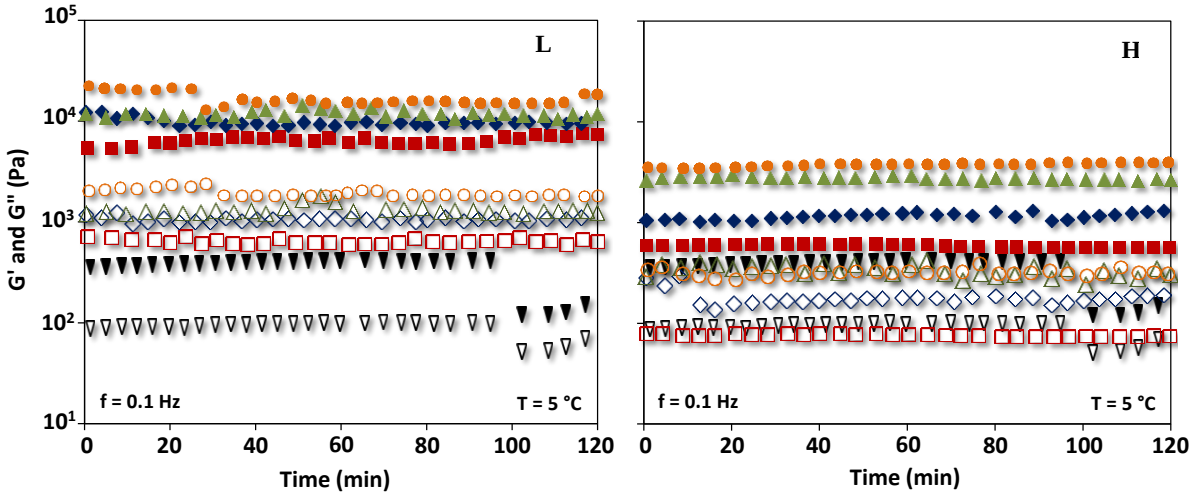

Figure 2.3 Time sweeps of mango puree with different gellan concentration, full symbols – storage modulus (G'), open symbols – loss modulus (G''); (▼) control, (■) 0.25 %, (♦) 0.5 %, (▲) 1.0 %, (●) 2.0 %.

After oscillatory time sweep at 5 ºC, mango puree samples texturized with gellan gum were analysed by stress sweep tests, performed at the same temperature. The dependence of the dynamic moduli with the applied stresses is presented in Figure 2.4.

When L gellan was used, the value of G' and G'' at the plateau corresponding to the linear viscoelastic region is around one decade higher than that of non-texturized mango puree. For gellan concentrations of 1.0 %, a decrease of G' at $\tau \sim 200$ Pa is observed, followed by a rise until the critical stress is reached (Figure 2.4). This behaviour seems to indicate a partial disruption followed by a recovery of the structure.

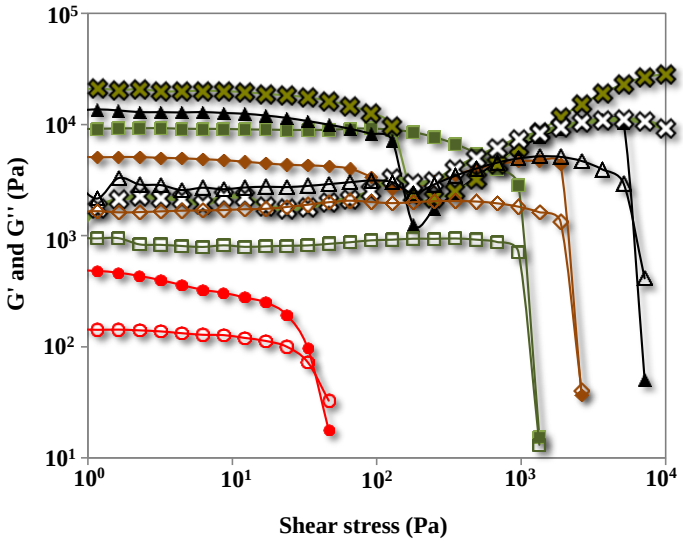

Figure 2.4 Stress sweeps of mango puree texturized with different low (L) acyl gellan concentrations performed after gel maturation at 5°C, full symbols – storage modulus (G'), open symbols – loss modulus (G"); (●)-0%, (♦)-0.25%, (■)-0.5%, (▲)-1%, (x)-2%.

The applied shear stresses may promote a partial disaggregation of the very compact and firm packing of the cross-linked double helices in the L gellan network, followed by an increment of the interpenetration of L gellan double helices, with each other or with the other macromolecules, present in the mango puree, resulting in a regain of the structure viscoelasticity (Mao, R. et al., 2000). Further studies, focused on the identification of the type and magnitude of the interactions taking place within the complex gellan/mango puree mixture (involving cations, sugars, organic acids, pectins and insoluble fibres, beyond the gellan molecules) are needed in order to understand this behaviour. For 0.25 and 0.5 %wt L gellan gum it can be noticed constant G' and G'' until the rupture of the structure takes place at around 1800 Pa and 1000 Pa, respectively.

The stress sweeps performed on the samples with H gellan have shown lower values of G' and G" within the linear viscoelastic region than those with L gellan, for the same biopolymer concentration (Figure 2.5). In addition, a well-defined plateau is observed for all H gellan concentrations tested, with a higher critical stress as the gellan concentration was increased. The partial disruption followed by a recovery of the structure is not observed in this case. As a result, the puree texturized with H gellan was able to resist to higher shear stresses without signs of its internal structure alteration.

Figure 2.6 shows the mechanical spectra of the mango puree (control) and texturized samples with different gellan concentrations. For the control, G' and G" increased with increasing frequency with a high frequency dependence, were G' values are greater than G" values through the whole range of frequencies studied. Mango puree may be characterized as having a behaviour normally observed for weak gels. This type of behaviour has been reported by other authors for mango pulp (Iagher et al., 2002).

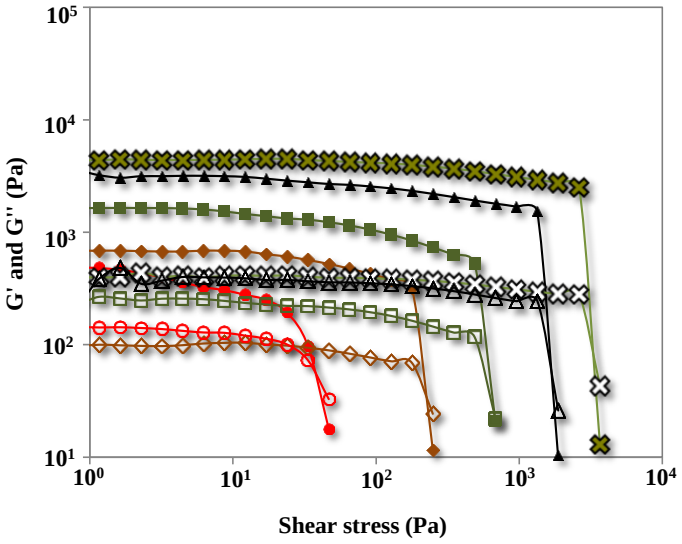

Figure 2.5 Stress sweeps of mango puree texturized with different high (H) acyl gellan concentrations performed after gel maturation at 5°C, full symbols – storage modulus (G'), open symbols – loss modulus (G''); (●)-0%, (♦)-0.25%, (■)-0.5%, (▲)-1%, (x)-2%.

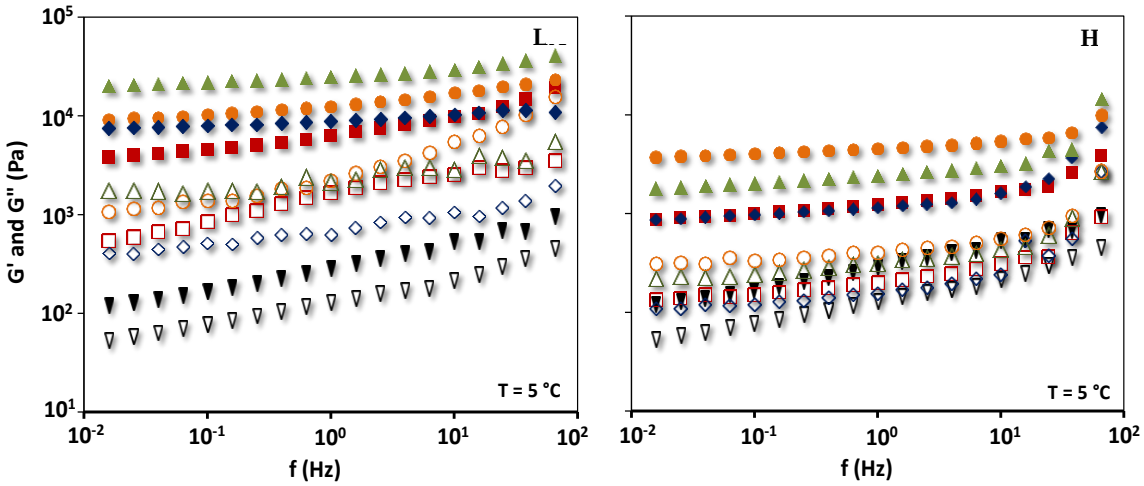

Figure 2.6 Frequency sweeps of mango puree with different gellan concentration, full symbols – storage modulus (G'), open symbols – loss modulus (G''); (▼) control, (■) 0.25 %, (♦) 0.5 %, (▲) 1.0 %, (●) 2.0 %.

In the presence of gellan, all formulations exhibited a gel spectra with G' always greater than G'' with a slight increase of the moduli as the frequency increased. In particular, both G' and G'' increased as L concentration increased, indicating a reinforcement of the gel structure. The frequency dependence of the dynamic moduli tended to decrease as the L concentration increased, which was quantified from the β exponent values obtained by fitting the power law regression to experimental data (Table 2.2). Similar behaviour was observed for samples with H gellan. For identical

concentrations of L and H, lower dynamic values were observed for H gels, which is consistent with the typical soft structure of these gels.

Table.2.2 β values of the power law regression for different gellan concentrations

Gellan (%wt)	L		H	
	β (G')	β (G")	β (G')	β (G")
0	0.23	0.23	0.23	0.23
0.5	0.16	0.21	0.06	0.09
1.0	0.10	0.12	0.10	0.09
2.0	0.11	0.22	0.04	0.10

Overall, the addition of either L or H gellan to the mango puree increased significantly the viscoelastic parameters, from the unstable and weak gel structure of the mango puree to a fully developed network with higher dynamic modulus and small frequency dependence.

Based on the mechanical spectra obtained for L or H /mango puree mixtures, the concentration of 1.0 %wt enables the formation of gels with better viscoelastic properties than those with 0.5 %wt gellan. The values of the moduli for 1.0 %wt of L/mango puree mixtures were slightly higher than 2.0 %wt L/mango puree, probably due an incomplete hydration of gellan gum when 2.0 %wt of L was used. The water and cations content of the puree, along with the time of heating at 90 ºC, may not have been enough to completely hydrate the polymer, with consequences on the viscoelastic properties of the gel formed. Therefore, the gellan concentration of 1.0 %wt was selected to study the effect of L/H blends on the viscoelastic properties of the texturized product.

Effect of L/H ratios on the gelation kinetics and viscoelastic properties of mango puree

A synergistic interaction exists between the native and deacylated gellan molecules upon gel formation when both gellan types are present in the system. Mao et al., (2000) reported that the weight ratio of L/H played a more important role in the texture properties of mixed gellan gels than the overall polymer concentration. Moreover, Morris et al., (1996) studied the L/H mixtures by Differential Scanning Calorimetry and observed two separate conformational transitions at temperatures characteristic of individual components. As such, there is the evidence of the establishment of zones within the gel network composed mainly by L or H molecules, connected to each other, enabling overall texture properties that cannot be obtained with only one type of gellan. In order to design a wider range of mango bar samples with dissimilar textural properties, formulations containing varied L/H ratios, maintaining constant the overall polymer concentration (1.0 %wt) were studied. The temperature sweeps of mango puree and L/H mixed gels (Figure 2.7) presented similar behaviour to those described with one type of gellan. All the formulations showed G' larger than G" in the whole temperature range studied. The initial gelation temperature for L/H gellan blends was

around 60 ± 1.1 °C for 75/25, 53 ± 1.3 °C for 50/50 and 50 ± 1.0 °C for 25/75. G' and G'' continued to increase with decreasing temperature reaching a maximum value equivalent to the final gelation temperature, 31 ± 1.1 °C for 75/25, 41 ± 1.0 °C for 50/50 and 40 ±1.1 °C for 25/75. The obtained gels have intermediate viscoelastic properties between those obtained with only L or H gellan.

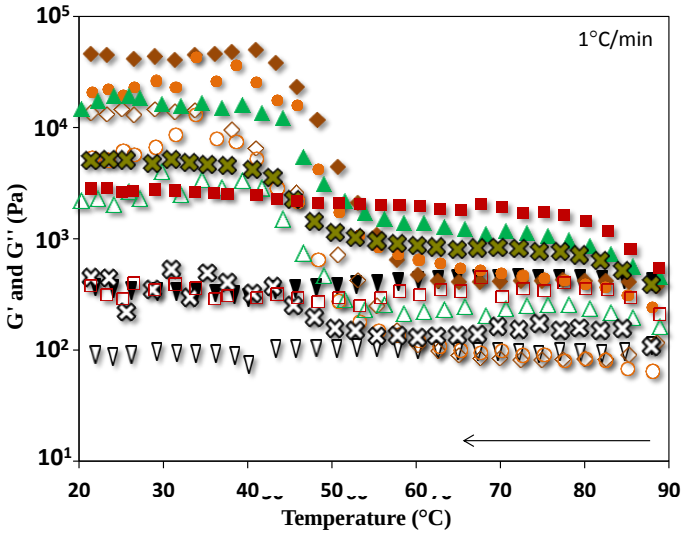

Figure 2.7 Cooling curve of mango puree with L, H and different L/H gellan ratios for a fixed overall gellan concentration (1.0 %wt); full symbols – storage modulus (G'), open symbols– loss modulus (G''); (▼) control, (♦) L, (●) 75/25, (▲) 50/50, (✖) 25/75, (■) H.

Time sweeps at T = 5 °C carried out immediately after the temperature sweeps are presented in Appendix A.1. All samples exhibited constant dynamic moduli during the entire time range studied showing rapid gel maturation.

Figure 2.8 shows the stress sweeps for samples texturized with 1.0%wt gellan. It may be seen that the value of the elastic modulus at the plateau, for $\tau < 100$ Pa, becomes higher as the L/H proportion changed from 0/100 to 25/75. For the rest of the proportions (50/50, 75/25 and 100/0), the elastic modulus is even higher with quite similar values among them. In addition, samples with the L/H mixtures of 50/50 and 75/25 have more resistance at higher shear stresses, without perceiving significant changes in the internal structure, as happened at $\tau > 100$ Pa when only L gellan was used (100/0).

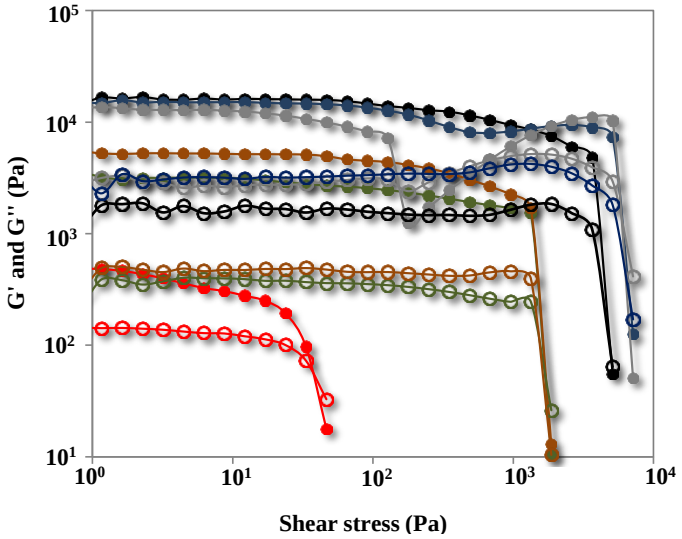

Figure 2.8 Stress sweeps for mango puree/gellan gum mixtures (1.0 %wt), at different L/H ratios performed after gel maturation at 5°C, full symbols – storage modulus (G'), open symbols – loss modulus (G"); (●) mango purée, (●) L/H 0/100, (●) L/H 25/75, (●) L/H 50/50, (●) L/H 75/25, (●) L/H 100/0.

Figure 2.9 shows the mechanical spectra of mango puree gels prepared with the L/H blends. The increase of L/H ratio led to the increase of both G' and G" while maintaining their slight dependency with the frequency, as illustrated by the small variation of β exponent values, $0.06 < \beta\ (G') < 0.09$ and $0.11 < \beta\ (G") < 0.13$ (Table 2.3). For all ratios used, texturized mango puree became less dependent with the frequency than the control. These mechanical spectra indicate that the obtained gels have intermediate viscoelastic properties between those obtained with only L or H gellan.

Table.2.3 β values of the power law regression for 1.0 %wt gellan at different L/H ratios

L/H ratio	β (G')	β (G")
Control	0.23	0.23
100/0	0.06	0.12
75/25	0.06	0.11
50/50	0.10	0.09
25/75	0.07	0.08
0/100	0.09	0.11

Pectic polysaccharides are one of the prominent structural constituents in the mango fruit (Tharanathan et al., 2006) and together with gellan gum can contribute to the stable and well developed structure for the texturized mango puree. The addition of both gellan gum types into the mango puree overcomes the thixotropic character of the mango puree.

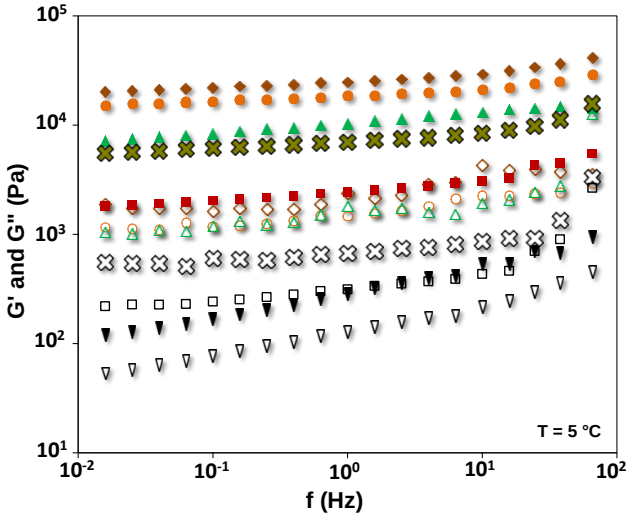

Figure 2.9 Frequency sweeps of mango puree with L, H and different L/H gellan ratios for a fixed overall gellan concentration (1.0 %wt); full symbols – storage modulus (G'), open symbols– loss modulus (G"); (▼) control, (♦) L, (●) 75/25, (▲) 50/50, (🞩) 25/75, (■) H.

Effect of different concentrations of the L/H 50/50 ratio on the viscoelastic properties of mango puree

To explore the full potential of mango puree and gellan, it was also important to study the behaviour of a specific L/H mixture at different overall gellan concentrations.

The evolution of storage and loss moduli with temperature for mango puree and L/H 50/50 at various concentrations is illustrated in Figure 2.10. For gellan concentrations of 0.25 and 0.50 %wt, the values of the dynamic moduli reached a maximum of 1 x 10^3 Pa and 2 x 10^3 Pa respectively. As the concentration of L/H 50/50 gellan increases from 1.0 to 2.0 %wt, the values of the dynamic moduli increase reaching 12 x 10^3 Pa and 17 x 10^3 Pa respectively. These temperatures sweeps have shown similar viscoelastic properties to those obtained in Figures 2.2 and 2.7. For all gellan concentrations studied, G' was always higher than G" in whole temperature range, this may be attributed to the formation of a strong internal network. Clearly, increasing the gellan concentration from 0.25 to 2.0 %wt leads to a reinforcement of the structure as it happened with the formulations when only L or H gellan was used.

The maturation kinetics at 5 °C were monitored throughout the evolution with time of G' and G" and are presented in Appendix A.2. For samples with gellan gum, both moduli were constant during the entire time range studied.

The results of the oscillatory stress sweep tests are presented in Figure 2.11. Gels prepared with 1.0 %wt and 2.0 %wt gellan have higher elastic modulus and resistance at higher shear stresses when compared to the gels produced with 0.25 %wt and 0.5 %wt. Furthermore, gels with 1.0 %wt and 2.0 %wt gellan presented a similar dependence of G' and G" with the shear stresses applied, which was also observed in Figures 2.5 and 2.6 for the gels with only L and H gellan.

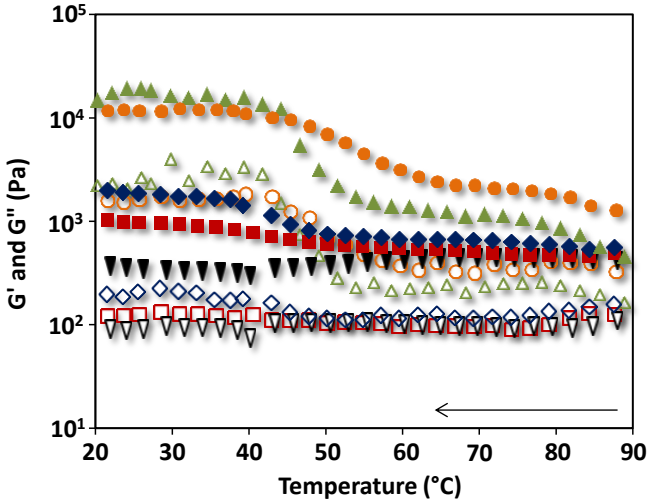

Figure 2.10 Cooling curve of mango puree with L/H gellan ratio of 50/50 at different concentrations; full symbols – storage modulus (G'), open symbols – loss modulus (G"); (▼) control, (■) 0.25 %, (♦) 0.5 %, (▲) 1.0 %, (●) 2.0 %.

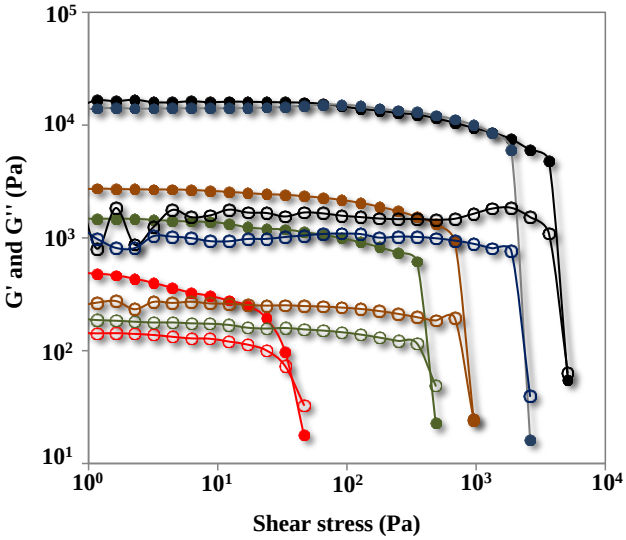

Figure 2.11 Stress sweeps for mango puree/gellan gum L/H 50/50 at various concentrations performed after gel maturation at 5°C; full symbols – storage modulus (G'), open symbols– loss modulus (G"), (●)-mango purée, (●) 0.25 %, (●) 0.50 %, (●) 1.0 %, (●) 2.0 %.

The mechanical spectra of the same texturized mango purée samples are shown in Figure 2.12. As the gellan concentration increased, higher values of G' and G" were obtained. Furthermore, it can be noticed a separation between dynamic moduli of 0.25 %wt and 0.5 %wt gels, from the ones produced with 1.0 %wt and 2.0 %wt. The β exponent values decreased from β (G') ~ 0.08 to 0.04 and β (G") ~ 0.14 to 0.1 (Table 2.4), for gellan concentrations from 0.25 to 2 %wt gellan, indicating an improvement of gel strength with increasing gellan concentration. The viscoelastic properties of

jellified mango puree samples prepared with all gellan concentrations studied, with a ratio of L/H 50/50, indicate the presence of structures close to strong gels.

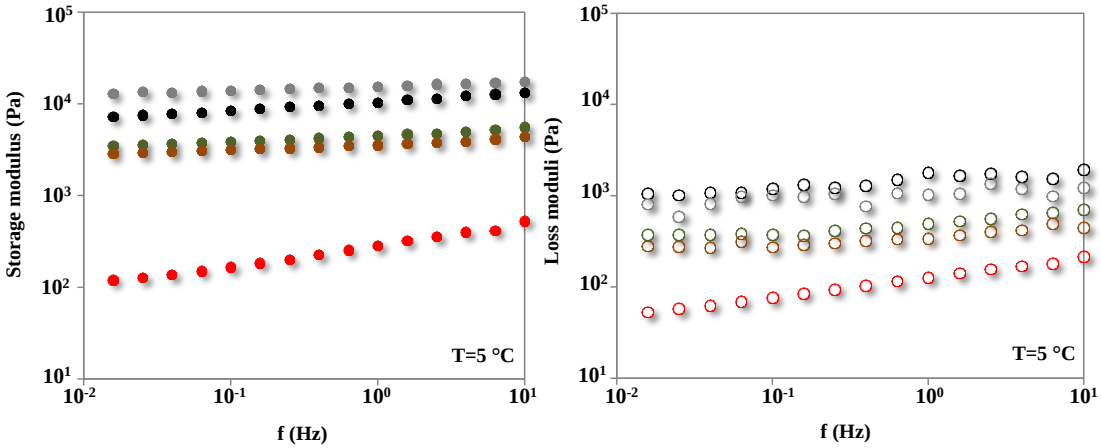

Figure 2.12 Mechanical spectra of mango puree texturized with L/H 50/50 gellan at different concentrations, (●)-0%, (●)-0.25%, (●)-0.5%, (●)-1.0%, (●)-2.0%.

Table.2.4 β values of the power law regression for 1.0 %wt gellan at L/H 50/50 at different concentrations

L/H 50/50 (%wt)	β (G')	β (G")
0	0.23	0.23
0.25	0.08	0.14
0.50	0.06	0.13
1.0	0.05	0.13
2.0	0.04	0.10

Overall, the results obtained from the small amplitude oscillatory shear measurements suggested that it is possible to create jellified mango bars, either by using different ratios of L/H gellan or L and H alone.

2.4.3. Confocal laser scanning microscopy observation

The structural organisation of the mango puree and mango bars was visualized by Confocal Laser Scanning Microscopy (CLSM). Microscopic observations of the mango puree with and without a fluorescent probe and that of stained mango puree/gellan gum with mixtures 1.0 %wt of L, H and L/H (75/25, 50/50 and 25/75) ratios were performed at 20 °C (Figure 2.13).

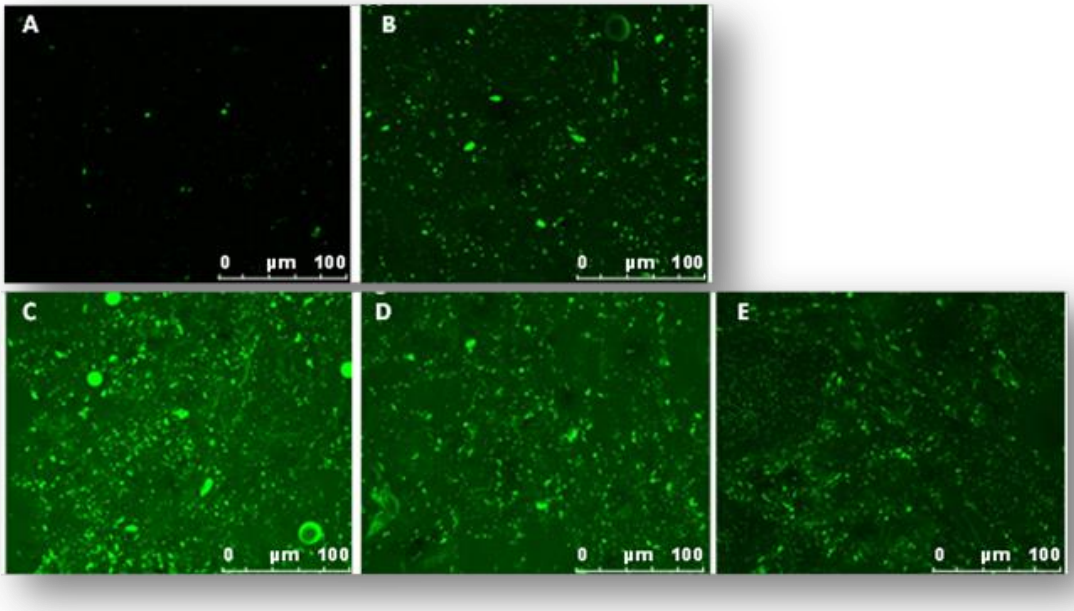

Figure 2.13 CSLM micrographs; mango puree (control): A - autofluorescence, B – containing non-covalently labelled fluorescein; mango purée with (1.0 %wt) gellan gum containing non-covalently labelled fluorescein C – Low acyl gellan, D - L/H 50/50, and E – High acyl gellan.

Mango fruit is rich in dietary fibre (DF), such as insoluble dietary fibre (IDF): cellulose, hemicellulose, lignin; and soluble dietary fibre (SDF) mainly pectin (Vergara - Valencia et al., 2007; Tharanathan, Yashoda, & Prabha, 2006). Many publications have confirmed that different plant living cells containing insoluble dietary fibre (IDF) might exhibit intrinsic fluorescence (Roshchina, 2003). Figure 2.13 A show the microstructure of unstained mango puree with relatively weak intrinsic fluorescence where less aggregates with a bright green colour can be detected corresponding probably to the insoluble dietary fibre (IDF). When fluorescein was added to the samples, a contrast by differences in fluorescence, with intense fluorescent zones equivalent to several insoluble carbohydrates, and less intense fluorescence zones, corresponding to the overall gel network of the mango bar, may be observed.

For samples with L, and, the mixture L/H 50/50, the distribution observed shows a similar pattern (Figure 2.13 C and D), resulting from the presence of aggregates surrounded by a continuous fluorescent phase, although there are large zones irregularly spread over the focal plane. This type of structure was also observed in the samples with formulations L/H 75/25 and 25/75 (results not shown) and represents a relatively homogeneous and well aggregated system.

The addition of only H gellan to the mango puree resulted in a major change in the structural organisation of the sample. Figure 2.13 E shows a less dense network of the fluorescent mango puree/HA sample, indicating a weaker gel structure. Figure 2.13 B, corresponding to the stained mango puree, also shows a weak, less structured and deficiently aggregated gel structure. These results

are in agreement with rheological measurements where lower values of G' and G" moduli were observed for these two systems. Clearly, the addition of L gellan to the mango puree, results in more homogeneous and well aggregated systems which corresponds to the desirable structure for the product under development.

2.5. Conclusions

In this chapter, the gel forming kinetics and gel properties of gellan gum/mango puree mixtures were studied. The results show that the chemical characteristics of the mango puree in terms of cations and water content, pH and TSS content are compatible with the hydration and gelation conditions required for the gelling agent. As referred in the literature (Bhattacharya, 1999; Iagher et al., 2002), mango puree shows a thixotropic behaviour, a decreasing of the moduli with the lapse of time is observed. The addition of L, H or L/H ratios to the mango puree improved its structure and produced texturized mango puree with interesting viscoelastic properties. Low maturation time (30 minutes) was needed for both L and H to reach a fully developed gel structure. For 1.0 %wt gellan concentration, L gellan forms stronger structures than H gellan. By using different L/H ratios for the same overall gellan concentration (1.0 %wt), it was possible to produce texturized mango puree samples with intermediate viscoelastic properties, with a progressive rise of the dynamic moduli as the L content increased. However, the overall gellan concentration of 1.0 %wt with L/H ratios of 50/50 and 25/75 are envisaged to be formulations producing mango bars with potentially more suitable viscoelastic properties.

CLSM observations also highlighted the formation of a denser structure for gels obtained with only L and L/H in different ratios, having the gels with only H a more loosened structure. The overall results obtained suggest that it is possible to create mango bars structures with interesting designed rheological and microstructural properties by using L/H gellan mixtures. For the final development of the mango bars, these properties still need to be correlated to inputs from texture analysis and sensory evaluation as well as further microscopy techniques at smaller distance scales in order to obtain more microstructural and morphological information about these systems.

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Chapter 3

Texture, microstructure, syneresis and consumer preference of gellan jellified mango bars

3.1. Summary

Two gellan types (low acyl-L and high acyl-H) used in specific L/H proportions (75/25, 50/50, 25/75) for an overall gellan concentration of 1.0 %wt were tested, in order to design different mango bars. The influence of the L/H gellan ratio on syneresis (passive diffusion and centrifugal dehydration), texture properties (Texture Profile Analysis and Stress Relaxation tests), microstructure (Cryo-Scanning Electron Microscopy) and texture sensory acceptance (Preference-Ranking test) was studied. The results obtained enabled to separate the bars in two groups: the ones showing greater hardness and brittleness, lower syneresis by passive diffusion and lower water holding capacity when subjected to external forces (only L gellan, L/H 75/25 and L/H 50/50). The second group are those presenting a softer structure with higher cohesiveness and springiness values, but higher syneresis by passive diffusion and better capacity to retain water when subjected to external forces (L/H 25/75 and only H gellan). The microstructure of mango bars was consistent with the syneresis and texture results. Those presenting higher hardness and lower syneresis by passive diffusion have shown a microstructure composed of a denser biopolymer network with lower pore size; while softer bars with higher springiness and higher syneresis by passive diffusion have shown larger pores and thin strings. From the Preference-Ranking test, the most appreciated mango bar in terms of texture was the one prepared with L/H at the ratio of 25/75.

3.2. Introduction

Tropical fruit production, trade and consumption have increased significantly, due to their attractive sensory properties and growing recognition of their nutritional and therapeutic properties (Bicas et al., 2011). Gorinstein et al., (1999), compared 8 tropical fruits and concluded that ripe mango had the highest gallic acid content and total polyphenolics, which make this fruit valuable for health maintenance, particularly protection against coronary heart disease and cancer. Its good texture, flavor and high amounts of antioxidants, mainly β-carotene, phenolic compounds, vitamin C and minerals (Schieber, Ullrich, & Carle, 2000), promoted mango as a suitable raw material for various applications in functional food production. One of its most processed forms is as pulp, which is further used for manufacturing final products such as fruit drinks, powders, jams, purees and dehydrated slices (Djantou, Mbofung, Scher, Phambu, & Morael, 2011; Sriwimon & Boonsupthip, 2011; Liu et al., 2012; Ledeker, Suwonsichon, Chamber, & Adhikari, 2014; Sogi, Siddiq, & Dolan, 2014).

Gellan gum, approved for use in food industry (Morris, Nishinari, & Rinaudo, 2012) was chosen in this work as texturizer. This hydrocolloid is used not only as a gelling agent; but also as stabilizing, film-forming, thickening and flavor-releasing agent (Bajaj & Singhal, 2007). Gellan gum has been used in a wide variety of food products including confectionary, jams, jellies, pie fillings, puddings, ice cream and yogurt (Noda et al., 2008).

Several factors may influence the texture of the gels formed, such as, type and concentration of gellan, pH, temperature, type of cations and their concentration, and sugar content. The advantages of using gellan gum for food product development rely on interactions with other polysaccharides and between the two gellan types, which allows the production of a wide range of textures (Chandrasekaran & Radha, 1995). Biopolymer blends may provide structures with superior properties than a system composed by only one type of polymer (Mao, Tang & Swanson, 2000; Banerjee & Bhattacharya, 2011).

Fruit purees/juices along with gelling agents can be used in the development of novel food products with a variety of textures (Mancini & McHugh, 2000). Banerjee, Ravi, & Bhattacharya (2013), developed gels based on gellan gum and agar with carrot juice. In addition, gels based on pineapple juice with different concentrations of gellan gum were studied by Saha & Bhattacharya (2010). Moreover, Sworn & Kasapis (1999) and Bayarri, Costell, & Duran (2002) studied the effect of sugar, a common additive in many food formulations, on gellan gels properties. The obtained food gels have been characterized on the basis of their texture attributes – one of the most important characteristics which may affect the consumer acceptance.

Texture attributes are used to monitor and control product quality and acceptability (Chen & Opara, 2013). In order to have a better understanding about the texture of the food gels, a characterization from a rheological point of view by employing small and large deformation tests (Zhang, Daubert, & Foegeding, 2005) might be suitable. Stress Relaxation tests (SR) involving small-

deformation, allow defining parameters such as relaxation time, elastic moduli and viscosity. Studies have been performed regarding stress-relaxation tests of food materials in order to investigate the effect of ingredients and processing conditions. Yildiz et al. (2013) analysed wheat starch/dietary fibre systems where different models were used to describe stress-relaxation behaviour, such as the Maxwell and Peleg models. Mancini, Moresi, & Rancini (1999) were able to describe the viscoelastic behaviour of several alginate gels, differing in the effective alginate concentration, by means of a generalized Maxwell model consisting of five elements. Furthermore, large-deformation tests such as texture profile analysis (TPA) partially simulate biting and chewing actions inside the mouth, are of practical use for product development and evaluation of consumer acceptance. From TPA analysis, hardness, cohesiveness and springiness are some of the most important parameters that should be assessed during food products development.

This chapter is focused on the relationship between the texture properties, microstructure, syneresis and sensory acceptance of mango bars, as convenient products presenting sensory attributes identical to the fresh fruit. The bars were produced using different L/H ratios, with the same overall concentration (1.0 % wt), enabling the design of a wide range of texture properties. TPA, SR tests, Cryo-SEM analysis, syneresis (passive diffusion and centrifugal dehydration) and sensory evaluation by Preference Ranking method were performed in order to select the formulation with the highest acceptability for the final mango bar product.

3.3. Materials and methods

Materials

Gellan gum and mango fruits where purchased as described in section 2.3.1. – Chapter 2.

3.3.1. Preparation of mango bars

Mango puree was prepared as described in section 2.3.2. – Chapter 2. Afterwards, the obtained puree was transferred into an 80 mL glass beaker immersed into a 1 L hot water bath. The goal was to improve the gellan dispersion avoiding the heat burn of the mango puree. The water bath temperature was increased in order to heat the mango puree up to a temperature of 88±2 °C followed by the addition of gellan powder and stirring at a rotation speed of 1640 xg with a four bladed impeller. MP (mango puree)/L/H samples with an overall gellan concentration of 1.0 %wt varying the L/H ratio (MP-L, MP-L/H75/25, MP-L/H50/50, MP-L/H25/75, MP-H), were prepared. The MP/gellan mixtures were placed into rectangular silicon moulds (W x H x L = 27 x 10 x 50 mm) and allowed to set at room temperature (22 ± 2 °C) (Figure 3.1). Afterwards, the samples were stored at 5 °C for 30 min before being analysed.

The detailed procedure for the selection of gellan concentration (1.0 %wt) is described in section 2.4.2. - Chapter 2. The short time of stirring (30 s) at 88±2 °C was chosen in order to promote the dispersion of the gellan powder, as well as enzyme deactivation (polygalacturonase, — responsible for texture softening and polypheniloxidase for browning) with minimal deterioration of the sensory properties of the fruit.

Figure 3.1 Mango bars used for syneresis, sensory analysis and texture measurements.

3.3.2. Syneresis

Passive diffusion

Syneresis (%) was determined using an analytical balance (Kern, Germany, sensitivity = ± 0.01 mg) by weighing the mango bars sample (W_1). Afterwards the samples were placed in a glass cell sealed with parafilm and stored at 5 °C. At regular intervals, the condensed water from the cell walls and samples surfaces was removed with tissue paper and the samples were weighed again (W_2). Syneresis (%) is expressed as the mass loss regarding the initial mass: $(W_1-W_2)/W_1x100$. Reported values are averages of 3 independent measurements.

Centrifugal dehydration

Rectangular samples with dimensions: W x H x L = 4 x 4 x 10 mm were placed in a centrifuge filter, (Fischer Scientific) with 0.2 μ pores and first centrifuged at various forces (200, 400, 600 and 800 x g) for 30 min. Afterwards, new samples were prepared and centrifuged at 600 x g for a scheduled time intervals between 10 to 120 min. The samples were weighed immediately after the centrifugation runs. Similar to passive diffusion tests, the weight ratio after and before centrifugation (W/W_0) was used to characterize the water holding capacity (WHC) of the mango bars. All measurements were made in triplicate.

3.3.3. Stress-relaxation tests

Stress Relaxation tests were carried out with a Texture Analyzer model TA.TX Plus (Stable Micro Systems, Ltd., UK) equipped with a 50 N load cell, using an aluminium flat plate with a diameter of 60 mm. Samples with dimensions of W x H x L =27 x 10 x 25 mm were transferred to the equipment. A thin layer of paraffin oil was applied between the equipment surfaces and the testing samples in order to avoid friction. The samples were subjected at different strains (5 and 10 %) and cross-head speeds (0.05 and 0.5 mm s^{-1}) and allowed to relax for 1200 s, while stress decay was monitored over time. Triplicate tests were performed for all the samples.

The Simplified Maxwell, Generalized Maxwell and Peleg models (described in section 1.4 – Chapter 1) were fitted to the experimental results and the goodness of the fits was evaluated using the correlation coefficient (R^2) and the Absolute Average Deviation (AAD) calculated by Equation 3.1.

$$AAD = \left\{\left[\sum_{i=1}^{n}\left(\frac{|y_{i,exp}-y_{i,calc}|}{y_{i,exp}}\right)\right]/n\right\} \times 100 \qquad \text{Eq. 3.1}$$

where $y_{i,exp}$ and $y_{i,calc}$ are the experimental and calculated responses, respectively, and n is the number of experimental runs (Rodrígues-Sandoval et al., 2009).

3.3.4. Texture Profile Analysis

In order to mimic the human biting action, the samples were analysed with a Texture Analyzer model TA.TX Plus (Stable Micro Systems, Ltd., UK) equipped with a 50 N load cell, using a method based on the one used by Mandala, Palogou, & Kostaropoulos, (2007). A double compression cycle test was performed up to 60 % strain of the original height using an aluminium plunger with 60 mm diameter. A time interval of 5 s was elapsed between the two compression cycles. A thin layer of paraffin oil was applied between the plates and the testing sample in order to avoid friction. Hardness (N) was defined as the maximum force achieved during the first cycle of compression and springiness was measured as the ratio between the second and first compression distances until maximum forces. Cohesiveness was calculated as the ratio of the positive force area during the second cycle to that of the first cycle of compression. All measurements were carried out at room temperature (20±2 °C) in triplicate.

3.3.5. Cryo Scanning Electron Microscopy (Cryo-SEM)

The method used was based on the one applied by Sriamornsak et al., (2008). Cryo-scanning electron microscopy (Cryo-SEM) of thin slices of mango bars with different L/H gellan ratio were performed with an EOL JSM 6301 F / Field Emission, Oxford UK instrument, equipped with a cryo sample preparation system (Gatan Alto 2500). The samples were immersed into liquid nitrogen at -210 °C for 15 sec. The mango bars samples were freeze fractured to expose fresh surfaces for sublimation,

then covered with a 10 nm layer of gold. The gold coated samples were observed and photographed with an accelerating voltage of 15 kV and at temperatures below -135 °C and a magnification in the range of 5000-40000 X.

3.3.6. Sensory analysis

Sensory analysis was performed in order to identify the mango bar with the highest texture acceptability. A Preference-Ranking test was performed (ISO 8587:2006) whereby each panellist was asked to evaluate the samples by preference, considering one single sensory attribute – the overall texture. This option was justified due to different textures among the mango bars, while the other attributes were considered relatively similar. Texture will be determinant in selecting the mango bars, taking into account that this attribute is one of the most important in developing food products similar to the bars. The sensory evaluation of the mango bars was carried out in a sensory room with 6 analysis boxes, in accordance with ISO 8589:2006. The 63 panellists (49 females and 14 males), aged between 20 and 65 years old, were students or staff members of Instituto Superior de Agronomia (Lisbon, Portugal) identified as regular consumers of mango fruit. The five different mango bars were presented at room temperature (20 – 22 °C) in random order and labelled with randomly-generated three-digit codes.

The score sheets required the judges to rank their five samples in the order of preference: (1) the least; (2) slightly; (3) moderately; (4) neither liked nor disliked and (5) the most preferred texture (Meilgaard, Civille, & Carr, 1999). Additionally, the panellists were asked to justify their choices. Friedman test (significance level $p \leq 0.05$), was used to determine whether each sample was significantly preferred over the others.

Statistical analysis

One-way variance analysis was performed to evaluate the statistical difference between estimated parameters of the samples. The software Scientist was used to fit the stress relaxation models to the experimental data. Principal Component Analysis (PCA) was carried out to study the multivariate results regarding the bars physical characteristics using the software STATISTICA version 6.0 (2001) (StatSoft Inc., Tulsa, OK, USA). PCA is a statistical technique used to identify the smallest number of latent variables, called principal components that explain the greatest amount of observed variability. The score vectors, which relate the individual experimental samples to each other, can be used as response variables to identify significant factors, while the loading vectors can be used to identify both the response variables describing the main variations and the response variables describing the same phenomena among the samples (Quinn & Keough, 2002).

3.4. Results and discussions

3.4.1. Syneresis

Syneresis of the formed gels is a natural phenomenon during which unbound excess water is released from the formed gel matrix. This section is focused on the evaluation of syneresis of mango bars with different L/H ratios by passive diffusion and dehydration by centrifugation.

Dehydration by passive diffusion

The determination of syneresis (%) by passive diffusion from mango bars with different L/H ratios was used to evaluate the ability of the structure to contain water inside, and maintain its physical characteristics during storage. Mao, Tang, & Swanson, (2001) also studied how the water holding capacity of gellan gels during storage was affected by Ca^{2+} concentrations and found that the water loss was only 1-2 %, independent of Ca^{2+} concentration. Figure 3.2 show the influence of storage time on the syneresis of different mango bar formulations. All the samples were stable during refrigerated storage (5 °C) with a minimum syneresis below 2 % for MP-L and a maximum of 8 % for MP-H in the last day of storage. Thus the formulations with higher content of H gellan show a higher percentage of water loss. However, the percentage of water loss can be considered minor since the initial water content of the mango puree is high (around 81 %).

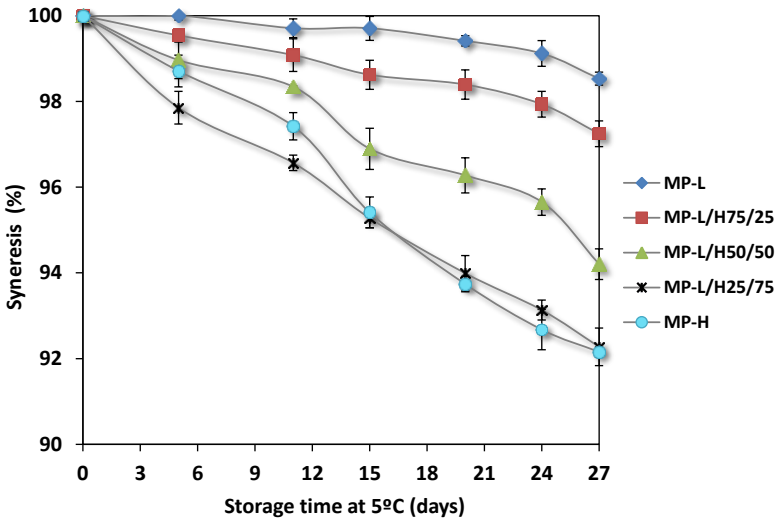

Figure 3.2 Influence of storage time on syneresis (water loss) of mango bars at different L/H ratios.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan).

Dehydration by centrifugation

The ability of the mango bars to withstand to the external force is shown in Appendix B and Figure 3.4. For all the L/H ratios, the mango bars present a rapid decrease in WHC with increasing centrifugal force from 400 to 800 x g (Appendix B). In addition, it can be seen that mango bars with high content of H gellan withstand better to the external forces. It is know that H gellan forms elastic gels, therefore, this behaviour might be explained by the elastic property of MP-H providing resistance to the external forces without any fracture from where water can be expelled.

Figure 3.3 shows the WHC of the mango bars when subjected to 600 x g centrifugal force at time intervals between 10 and 120 min. A large decrease of the WHC with centrifugation time is noticed. For samples MP-L, MP-L/H75/25 and MP-L/H50/50, WHC presents an abrupt decrease at 40 min of centrifugation, followed by a gradual one. Samples MP-L/H25/75 and MP-H also show a gradual decrease of WHC with increasing time centrifugation. However, the sample MP-H exhibited the highest WHC for all time intervals of centrifugation. These results may be explained by the change in mango bars texture with varying L/H ratios. When no external forces are applied, brittle structures such as MP-L or MP-L/H75/25 have a lower syneresis, but when subjected to centrifugal forces, fracture may occur and thus the water is more easily expelled.

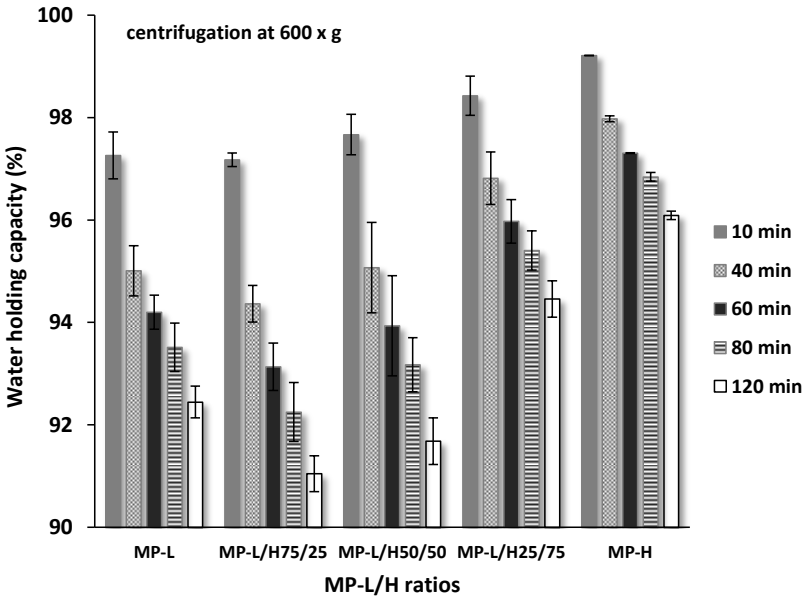

Figure 3.3 Influence of centrifugation time on water holding capacity of mango bars at different L/H ratios.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan)

3.4.2. Stress relaxation properties of mango bars

The relaxation curves with an imposed strain of 5 % at both cross-head speeds (0.05 and 0.5 mm s^{-1}) did not discriminate the samples (Appendix C – Figures C.1 and C.2). The relaxation curves with an imposed strain of 10 % at a cross-head speed of 0.05 mm s^{-1} were also very difficult to discriminate (Appendix C – Figure C.3). As such, the relaxation curves selected to fit to Simplified Maxwell, Generalized Maxwell and Peleg models (see section 1.4 - Chapter 1, Equations 1.1, 1.2 and 1.3) where those with an imposed strain of 10 % and a cross-head speed of 0.5 mm s^{-1} represented in Figure 3.4. A maximum stress was observed for all cases, followed by a typical behaviour of viscoelastic materials (biofilms, gels and certain foods), where the stress necessary for the maintenance of the deformation decreased with time (Tang, Tung, & Zeng, 1998; Lui & Peng, 2007; Pereira et al., 2013; Roopa & Bhattacharya, 2014). The highest stress values were observed for MP-L sample. As the L gellan content decreased, lower values of the stress were observed over the entire time range studied, with a good discrimination between the different bars formulations.

The formulation MP-H registered a considerable lower initial stress (σ_0=0.5 kPa). The reason for this behaviour is due to different properties of H gellan which produces soft, elastic and non-brittle gels. These characteristics tend to be present in the mango puree/H gellan matrix, and when it is subjected to a constant strain, very short relaxation times are observed, with an almost constant stress-time curve during the entire test. These results indicate that H gellan imparts a lower structural firmness to the bars than L gellan. Nevertheless, MP-H sample is a viscoelastic material, as observed in previous rheological measurements (Chapter 2 – Section 2.4.2). The mechanical spectrum of this sample has shown values of the elastic modulus (G') greater than the viscous modulus (G'') at all values of frequency.

The relaxation curves were fitted by three models: the Peleg's model, the simplified Maxwell model and generalized Maxwell model (with two Maxwell elements in parallel with a pure elastic element). A typical relaxation curve (formulation MP-L) fitted with the three models is presented in Figure 3.5. The generalized Maxwell model with two Maxwell elements was the one that best described the experimental data for all bars formulations, with a correlation coefficient of 0.999 and AAD values in the range of 0.06-0.1. The respective estimated parameters values for all mango bars samples are presented in Table 3.1.

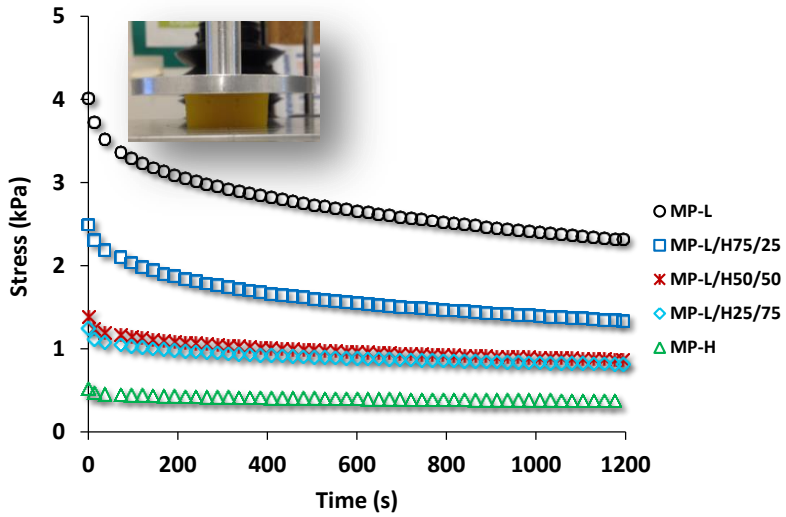

Figure 3.4 Stress relaxation curves of MP-L/H samples for 10 % strain and 0.5 mm s^{-1} cross-head speed.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan)

The moduli of elasticity (E_1, E_2) quantify the rigidity of the material, therefore, the materials with the highest elastic moduli values are the firmest (Rodrígues-Sandoval, Fernandez-Quintero, & Cuvelier, 2009). For viscoelastic solids, the stress does not completely relax but settles to an equilibrium value after a certain time. When the viscous flow of the material is exhausted the rearrangements stop and no further energy is dissipated. The elastic response persists and it is characterized by the equilibrium elastic moduli (E_e), thus indicating the elastic nature of the material (Cespi et al., 2007). The sample MP-H has a value of E_e lower than of the other samples, meaning that almost all the energy was dissipated during relaxation, leading to a softer network at relaxation equilibrium. As observed for E_1 and E_2, E_e values also decreased with increasing H gellan proportion. Nussinovitch, Peleg, & Normand, (1989) observed a similar correlation between E_e and firmness for agar and alginate gels, where softer gels presented lower E_e values.

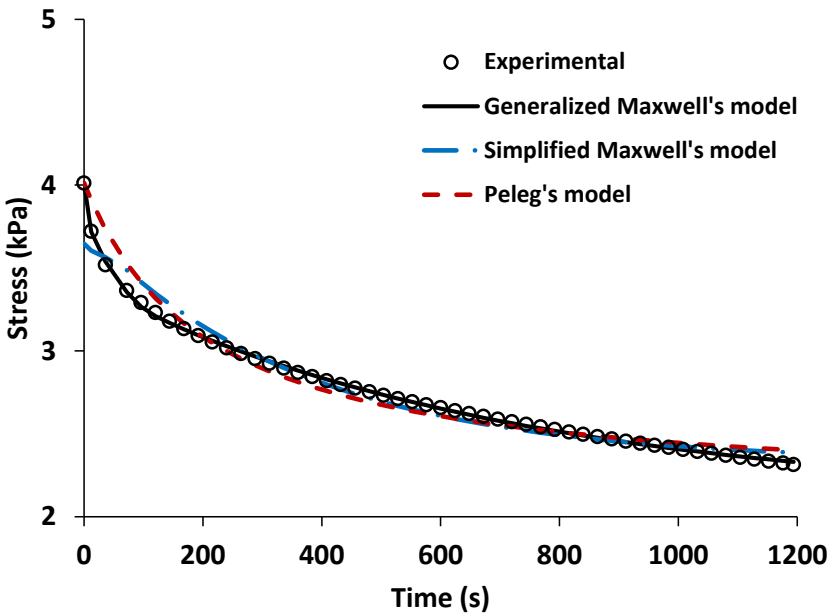

Figure 3.5 (○) Experimental stress relaxation data for sample MP/L; the lines represent the fittings with: generalized Maxwell's model with two elements, simplified Maxwell's model and Peleg's model.

Relaxation times (λ_i) and the dashpot components (η_i) of the model decreased as the H gellan content increased. All the formulations presented a typical solid-like behaviour, as the relaxation time for the first element varied between 500 - 700 s (Roopa & Bhattacharya, 2014). The viscosity parameter (η) is in accordance with the moduli and relaxation times values. Samples with higher content of L gellan have a stronger network revealing higher viscosity and relaxation times values for the first and second elements.

The trend of all relaxation parameters obtained for the generalized Maxwell model with two elements indicates that the variation of the L/H ratio affects significantly the texture of the mango bars. Different textures, from firm, with high relaxation times (composed mainly by L gellan), to soft with lower relaxation times (composed mainly by H gellan) were produced.

Table 3.1

Viscoelastic parameters of generalized Maxwell model fitted to SR data using Equation 1.2 (section 1.4 - Chapter 1) for mango bars jellified with L and H gellan and different L/H gellan ratios

Formulations	E_1 (kPa)	E_2 (kPa)	E_e (kPa)	λ_1 (s)	λ_2 (s)	η_1 (x 10^3 Pa s)	η_2 (x 10^3 Pa s)	R^2	AAD (%)
MP-L	13.2 ± 0.8^a	5.7 ± 0.6^a	21.0 ± 0.9^a	683.7 ± 1.8^a	50.9 ± 0.2^a	9028.1 ± 0.4^a	288.7 ± 0.3^a	0.998	0.094
MP-L/H75/25	8.6 ± 0.3^b	4.0 ± 0.5^a	12.2 ± 0.5^b	625.7 ± 1.6^b	31.7 ± 0.1^b	5353.6 ± 0.4^b	127.8 ± 0.2^b	0.999	0.151
MP-L/H50/50	3.6 ± 0.3^c	2.1 ± 0.2^b	8.2 ± 0.2^c	616.2 ± 1.4^b	27.7 ± 0.1^c	2231.8 ± 0.1^c	54.8 ± 0.2^c	0.999	0.101
MP-L/H25/75	2.8 ± 0.1^d	1.8 ± 0.1^b	7.8 ± 0.2^c	600.9 ± 1.6^b	28.1 ± 0.1^c	1685.4 ± 0.1^d	51.0 ± 0.1^c	0.999	0.103
MP-H	0.9 ± 0.1^e	0.7 ± 0.1^c	3.6 ± 0.1^d	593.4 ± 1.5^b	27.8 ± 0.1^c	557.3 ± 0.4^e	18.5 ± 0.1^d	0.998	0.062

Means of three replicates ±SD. One-way variance analysis was performed to evaluate the statistical difference between fitting parameters. Values in the same column followed by different superscript letters differ significantly ($p < 0.05$).

MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan).

3.4.3. Texture Profile Analysis

Figure 3.6 summarizes the TPA parameters, obtained upon compression of 60% of the initial sample height, as affected by the different ratios of L/H gellan. The results show that the sample MP-L exhibited a similar hardness value to that of the samples MP-L/H75/25 and MP-L/H50/50 (Figure 3.6a). As the H gellan content further increased, a marked decrease of the hardness value was noticed for samples MP-L/H25/75 and MP-H. It is known that L gellan forms harder and more brittle gels than H gellan, either in simple or complex aqueous mixtures (Philips & Williams, 2009). Consequently, a maximum value of hardness could be expected for the sample MP-L, which was not observed. This behaviour may indicate that the addition of H gellan up to 50% of the overall gellan concentration is not sufficient to affect substantially the firmness conferred by the L gellan to the overall mango puree/gellan structure. Another hypothesis to explain this behaviour may be the presence of heterogeneous structures with more firm and/or softer regions in the matrix. However, the overall texture of the mango bars is significantly changed when H gellan increases, i.e., for the samples MP-L/H25/75 and MP-H a decrease of their hardness is noticed. The springiness increased with increasing the H gellan content, with a maximum (around 0.9) observed for the MP-L/H25/75 sample (Figure 3.6b). This means that the later formulation enables the production of mango bars with the higher elastic recovery when the compressive force has been removed. The value of the cohesiveness (Figure 3.6c) is quite similar for the formulations MP-L and MP-L/H75/25, presenting a substantial increase as the H acyl content increased, with a maximum for the sample MP-H (0.6). Cohesiveness is related to how well the structure of a product withstands compression, so that, if a hard product maintains its internal structure without failures when subjected to compression, it would also present a high cohesiveness. In this work it is observed a decrease of cohesiveness with increasing the bars hardness. This fact may be related to the observed samples fracture in the first compression, which is more pronounced in the firmer samples, with higher L gellan content. The samples containing mostly H gellan, withstood the first compression with fewer changes in their softer structure, leading to higher values of cohesiveness.

From the TPA force-time curves illustrated in Appendix D, it is shown that all the samples differentiate from each other by the magnitude level and shape of the curve. Brittle samples (MP-L, MP-L/H75/25 and MP-L/H50/50) present a lower magnitude of the second compression peak as compared with the first compression peak, while the elastic samples (MP-L/H25/75 and MP-H) showed a similar shape of the TPA curve, where first and second compression peaks were comparable.

The TPA results obtained under the conditions tested, demonstrates that the studied mixtures may be divided into two distinct groups: MP-L, MP-L/H75/25 and MP-L/H50/50 samples, are significantly harder, with lower cohesiveness and springiness than the samples MP-L/H25/75 and MP-H.

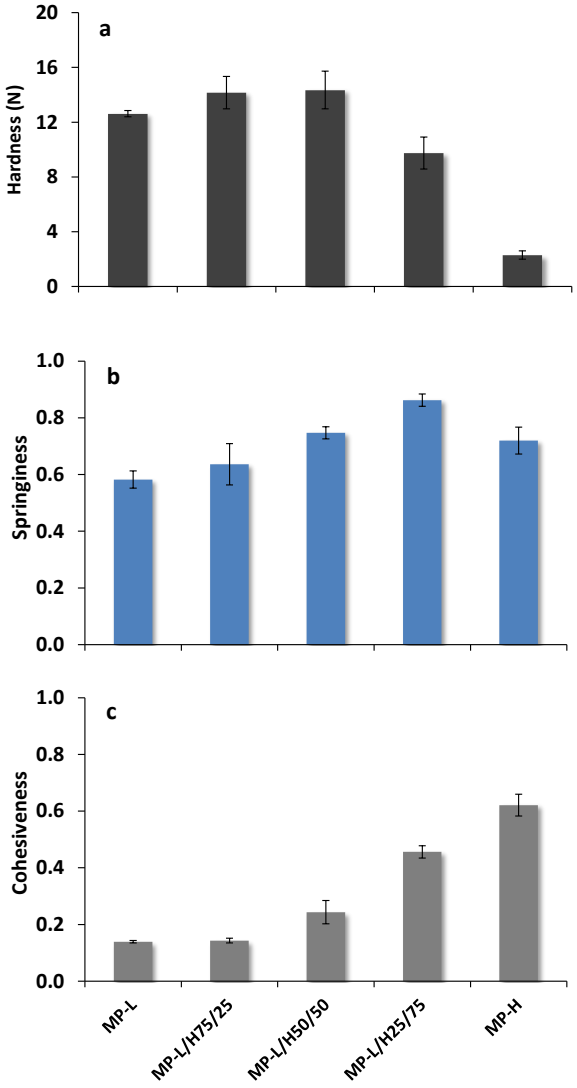

Figure 3.6 TPA parameters of 1.0 %wt MP-L/H samples: (a) hardness, (b) springiness and (c) cohesiveness.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan)

3.4.4. Cryo-SEM observations

In Figures 3.7 and 3.8 are presented images of the samples MP-L, MP-L/H50/50, MP-H and MP, at lower and higher magnification, respectively. The microstructure of the samples consists of a complex network made of gellan molecules together with the insoluble and soluble fibres from the mango puree holding water inside. The interaction between MP constituents and different L/H blends resulted on different average pore after water sublimation. As can be observed in Figure 3.7a, the

sample MP-L shows a continuous and dense network. By the contrary, the sample MP-H (Figure 3.8c) presents a biopolymer network with a much higher average pore size.

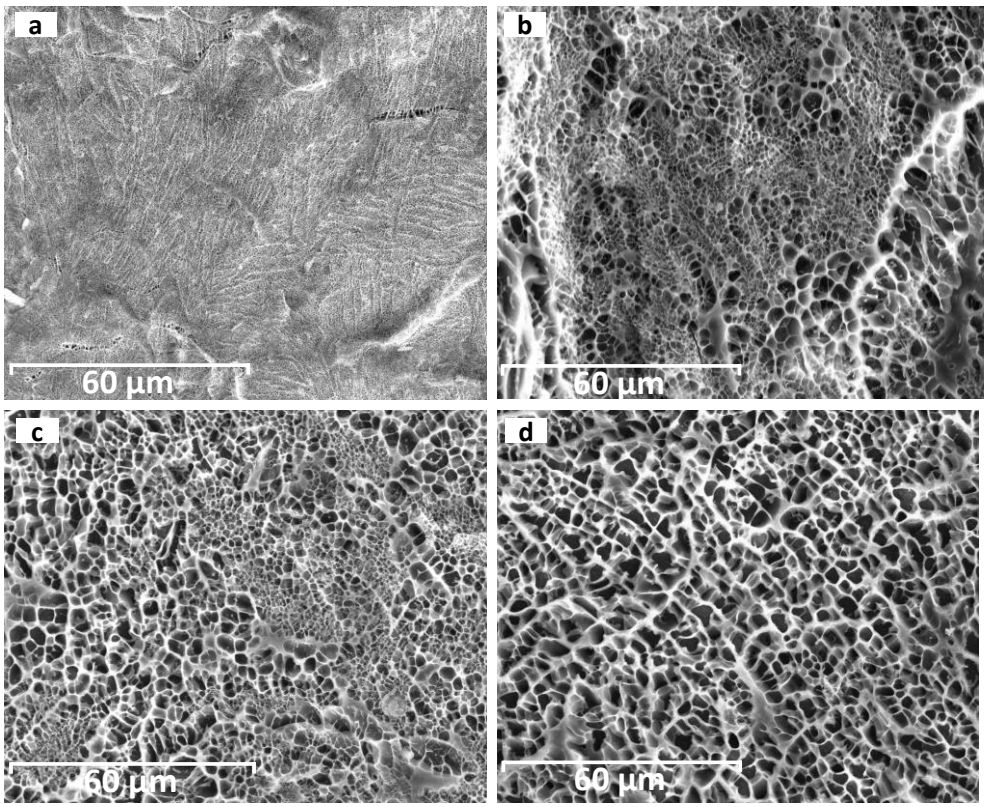

Figure 3.7 CryoSEM images with lower magnification: (a) MP-L, (b) MP-L/H50/50, (c) MP-H, (d) MP.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan)

The mango bar with formulation MP-L/H50/50 shows intermediate properties, consisting on a porous structure with an average pore size lower than that of the sample MP-H (Figure 3.7b). This behaviour may be seen more clearly on CryoSEM images with higher magnifications (Figure 3.8). Samples MP-L, MP-L/H75/25 and MP-L/H50/50 displayed a denser structure with numerous pores of medium to small size, in the range between 0.1 and 0.5 µm (Figures 3.8a, 3.8b and 3.8c), while the samples MP-L/H25/75 and MP-H show the formation of larger pores with thin strings (Figures 3.8d and 3.8e). In addition, MP-H samples show a porous structure similar to MP, with pore size up to 10 µm (Figure 3.8f).

Beyond pore size, differences in homogeneity are observed, especially in the image with lower magnification (Figure 3.7d), where MP sample displays a more homogenous microstructure. When gellan is added to the puree, independently of the L/H ratio, a heterogeneous structure is formed,

showing different regions with small and large pores coexisting in the matrix. This fact may be due to different local chemical composition (e.g. pH, ionic strength, specific cations) that can influence the gel formation and structure. In conditions where there are enough cations and a pH value near the isoelectric point, the electrostatic repulsions are suppressed, allowing an extended aggregation of the helices, originating a more compact structure (Turgeon, Beaulieu, Schmitt, & Sanchez, 2003). A similar behaviour was observed by Kiani, Mousav, Razavi, & Morris, (2010) who studied the effect of gellan alone and in combination with high-methoxl pectin on the structure of yogurt-based drink. The authors confirmed by confocal laser scanning microscopy that the structure becomes less homogeneous with the incorporation of gellan and high-methoxy pectin to the yogurt drink.

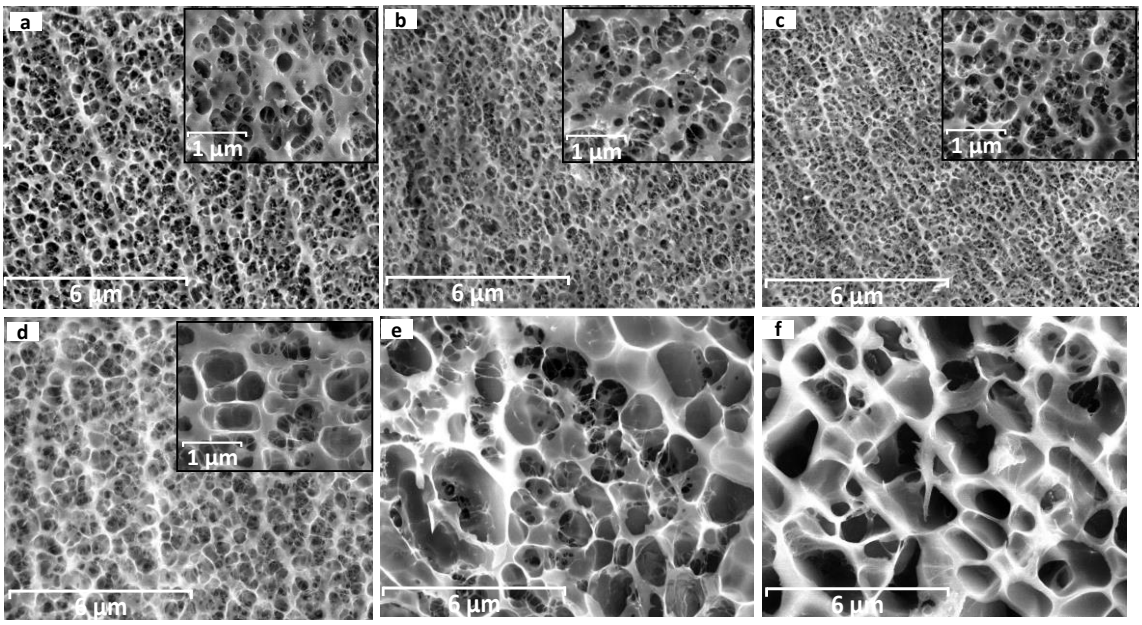

Figure 3.8 CryoSEM images with higher magnification: (a) MP-L, (b) MP-L/H75/25, (c) MP-L/H50/50, (d) MP-L/H25/75, (e) MP-H, (f) MP.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan)

The microstructure characteristics observed by Cryo-SEM analysis may be related to the syneresis and texture parameters shown in the previous sections. The denser microstructure given by higher L gellan content enables a higher resistance to deformation, conferring greater hardness values to the bars samples, resulting eventually on a broken gel structure for the applied deformation in TPA tests. As expected, denser microstructures present a lower syneresis by passive diffusion, where the water was maintained inside the small pores. When subjected to external force, fracture may have occurred and the water was released easily. As the H gellan content increased, the pore size of the gel

structure is greater, conferring the structure less resistance to the applied compression forces, with a higher elastic recovery as shown by the springiness values and, simultaneously, higher syneresis by passive diffusion. Still, this structure presents a higher capacity to maintain the water inside when subjected to centrifugal forces.

3.4.5. Multivariate analysis of mango bars physical parameters

To obtain an overview about the effect of L/H gellan ratios on the syneresis and texture properties of fresh fruit mango bars, a study of the multivariate data was performed. PCA was conducted with respect to the mean values from syneresis, TPA and SR parameters. The two principal components (PC1 and PC2) accounted for 95.6 % of the total variance, (Figure 3.9). PC1 explained 82.11 % of the total variance with the passive diffusion during 27 days of storage, stress-relaxation variables (λ_I, E_i, η_i) and hardness, grouped on the positive side of X-axis indicating high correlation with more firm samples composed of higher levels of L gellan (MP-L and MP-L/H75/25). The component PC2 correlated to all the dehydration by centrifugation variables, springiness and cohesiveness and separates the bars with only one type on gellan from those produced by L/H blends.

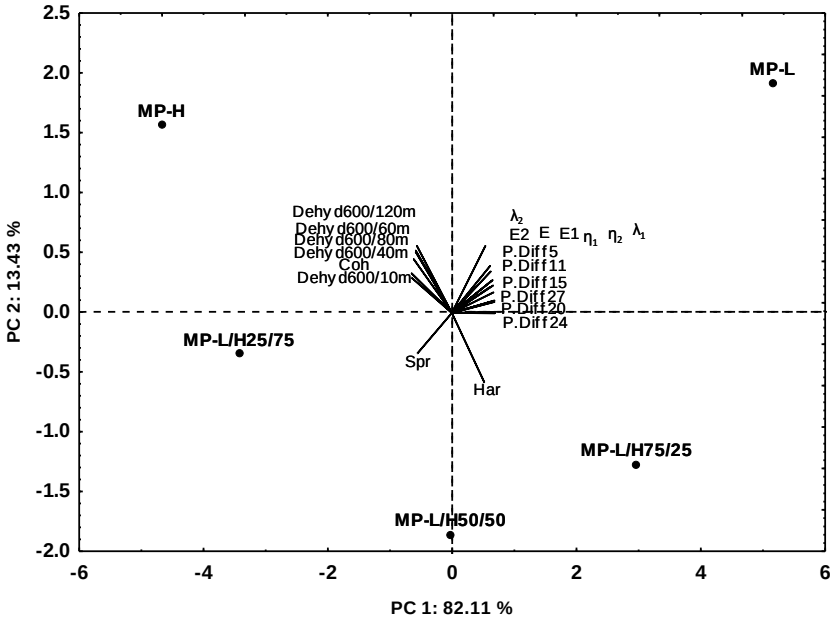

Figure 3.9 PCA biplots of variable coordinates of textural parameters for mango bars with different L/H ratios. P.Diff (5, 11, 15, 20, 24, 27): Passive Diffusion from each day of storage; Dehyd (200g, 400g, 600g, 800g): Dehydration by centrifugation at several centrifugation forces; Dehyd600/(10m, 40m, 60m, 80m and 120m): Dehydration by centrifugation at 600 x g at several time intervals; E_e: equilibrium elastic moduli (Pa); E_1 and E_2: elastic moduli (Pa); λ_1 and λ_2: relaxation times (s); η_1 and η_2: Viscosity (Pa s); Har: hardness (N); Spr: springiness; Coh: cohesiveness.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan).

3.4.6. Sensory evaluation

The preference distribution for all five mango bar formulations is presented in Figure 3.10. The sample MP-L/H25/75 was significantly preferred over the others ($p < 0.05$), with 43 % of the panellists ranking it as having the most preferred texture (rank - 5). The sample MP-H was found to be the least preferred ($p < 0.05$), more than 50 % of the panellists did not appreciate it. This was justified, by almost 40 % of the panellists, by its high adhesiveness and soft texture. Results from Friedman test showed that there are no significant differences between the samples MP-L, MP-L/H75/25 and MP-L/H50/50 (Table 3.2). The preferred formulation, MP-L/H25/75, has been selected by the panellists due to more pleasant texture perception.

Considering the preferred formulation, MP-L/H25/75, it can be concluded that the low proportion of L gellan might be enough to impart the suitable firmness to the product, while H gellan, responsible for softness, imparts a well-balanced texture to the mango bar.

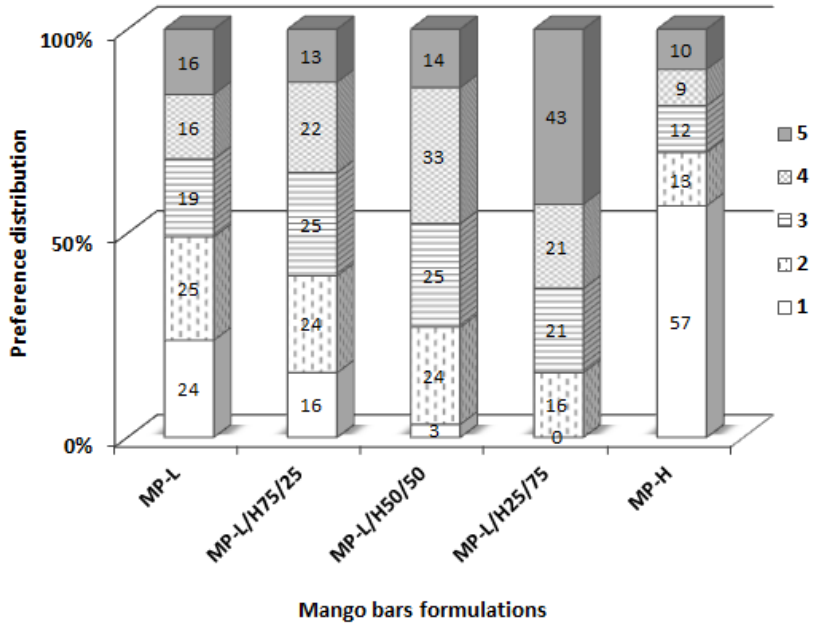

Figure 3.10 Preference distributions for mango bars formulations at different L/H ratios. Ranks: (1) the least; (2) slightly; (3) moderately; (4) neither liked nor disliked and (5) the most preferred texture.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan).

Table 3.2

Results obtained with Friedman variance analysis

Attribute texture sum of ranks (Friedman)	
Sample	Sum of ranks
MP-L	205
MP-L/H75/25	194
MP-L/H50/50	169
MP-L/H25/75	132
MP-H	241
Friedman number: 32.52, $p < 0.05$	
Critical Friedman number (ISO 8587:2006): 9.49	

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan).

3.5. Conclusions

Fruit bars produced with mango puree jellified with gellan gum at an overall concentration 1.0 %wt varying the Low acyl/High acyl gellan ratio (L/H) were characterised in terms of syneresis, instrumental texture, microstructure and texture sensory acceptance. Syneresis, stress-relaxation tests and texture profile analysis enabled to group mango bars into two different texture profiles:

(i) Bars that have a higher hardness and lower syneresis by passive diffusion, present a lower capacity to retain water when a centrifugal force was applied (only L gellan and L/H ratios of 50/50 and 75/25);

(ii) Bars with a softer, cohesive structure and higher elastic recovery upon removal of compression force present a higher syneresis by passive diffusion and better capacity to retain water when centrifuge force was applied (only H gellan and L/H at the ratio of 25/75).

The texture characteristics presented a good correlation with the microstructure observed by cryo-scanning microscopy. While mango bars presenting higher hardness have shown a denser biopolymer network with lower pore size; for softer bars with higher springiness, larger pores and thin strings were observed. From sensory evaluation, the preferred mango bar formulation in terms of texture was the one prepared with L/H ratio of 25/75. Further studies will be carried out with the selected formulation in what concerns product packaging and conservation.

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Chapter 4

Optimization of gellan gum edible coating for ready-to-eat mango bars

4.1. Summary

The optimisation of an edible coating based on low acyl (L) /high acyl (H) gellan gum for ready-to-eat mango bars was performed through a central composite rotatable design (CCRD). The independent variables were the concentrations of Ca^{2+} and gellan in the coating solution, as well as the storage time after coating application. The response variables studied, evaluated after coatings application, were coating thickness, mango bars firmness, syneresis, and total colour difference. Rheology studies (steady shear and dynamic oscillatory measurements) of coating solutions with different formulations provided important information related with their apparent viscosity and sol-gel transition. The selected formulation was the one containing 1 %wt gellan (L/H90/10) and 6 mM of Ca^{2+}. The release of eight volatile compounds from the uncoated and coated mango bars with the selected formulation was analysed by Headspace - Solid Phase Micro Extraction-Gas Chromatography (HS-SPME-GC) during nine days of refrigerated storage.

Results showed that the coating can improve the mango bars appearance and firmness, and may reduce syneresis and volatiles loss during storage.

4.2. Introduction

Ready-to-eat fresh fruit products became an integral part of a healthy life style throughout the world, particularly, due to their characteristics of freshness, potential health benefits, low caloric content and convenience (Raybaudi-Massilia, Mosqueda-Melgar, & Martín-Belloso, 2008). Food grade hydrocolloids (gelling and thickening agents, emulsifiers, stabilizers, etc) can interact with fruits and vegetables allowing the production of novel textures, an area of knowledge with great interest for food scientists. In Chapter 2, the first studies for the development of a fresh mango bar product were described, were the gel forming kinetics of mango puree/gellan gum mixtures and the viscoelastic properties of the gels formed were studied for different low acyl (L) / high acyl (H) gellan ratios and overall gellan concentration. The results obtained were correlated with inputs from texture analysis, sensory evaluation and microstructure in Chapter 3, which enabled the selection of one mango bar formulation.

Several authors (León & Rojas, 2007; Rojas-Argudo, del Río, & Pérez-Gago, 2009; Lima et al., 2010) analysed the potential of edible coatings to extend the shelf-life of fresh-cut fruit and vegetables. Considering that an edible coating can increase the commercial value of the mango bars, by acting as an effective preservation technique but also by improving appearance, firmness and syneresis, the development of a compatible coating and its optimization was performed.

Another important aspect when working with fruits and fruit products is their pleasant and characteristic flavour due to their content on volatile aroma compounds. During processing, the aroma compounds should be retained in the products as much as possible, as it is an important quality factor that influences consumer acceptability. The volatile compounds that are involved in the fruit flavour are produced through metabolic pathways during ripening, harvest, post-harvest and storage, and depend on many factors related to the variety and type of technological treatments (Ibáñez, López-Sebastián, Ramos, Tabera, & Reglero, 1998).

In the case of mango, aroma is formed by a complex mixture of compounds (more than 270 volatiles). Some authors consider terpenes, especially 3-carene, as the most important aroma constituents, due to the high percentage in the volatile fraction (50–60%) (Andrade, Maia, & Zoghbi, 2000), being described as a typical aroma of mango and tree leaves. The terpene hydrocarbons are considered to be important contributors to the flavour of several mango varieties, such as “Keitt”, “Kent”, “Tommy Atkins” and “Palmer” (Malundo, Baldwin, Ware, & Shewfelt, 1997). Besides terpenes, alcohols and aldehydes contribute to the typical flavour of mango (Liu et al., 2013). It has been reported in the literature that esters are the second most abundant compounds associated with the fruity aroma. Some mango cultivars have lactones and furanones compounds which impart an aroma similar to peach and pineapple, but cannot be detected in all the cultivars (Malundo et al. 1997). Monoterpenes, lactones, aldehydes and esters are the main groups of compounds that give the characteristic aroma of the mango fruit. However, mango cultivars have a wide range of aroma

compounds, so it is expected to have considerable variation of volatiles, as can be seen in Table 4.1 (Pino et al., 2005).

Since the contribution of volatile compounds to the final aroma depends on whether the concentration of the food products is above the perception threshold, OAVs (Odour Activity Values) were introduced to determine impact odorants. OAVs are calculated as the ratio between the concentration of individual substance in a sample and the threshold concentration of this substance (odour threshold value, the minimal concentration that can be detected by human). OAVs calculation depends both on measuring concentration and on odour threshold in the same matrix. Only those odorants with OAV > 1 can be perceived. When OAV < 1, it means that the volatile concentration is below the detection limit and, as a consequence, has a low contribution to the final aroma of the fruit (Pino & Mesa, 2006). Table 4.1 presents the OAVs for some volatile compounds present in mango fruit and, as can be noticed, lactones, esters and aldehydes can reach an OAV up to 4. The diversity of volatile compounds present in the mango fruit is due to the characteristics of the cultivars, the degree of ripeness, and type of analysis or processing conditions (Pino & Messa, 2006). Moreover, the studies on the volatile compounds for *Palmer* variety from Brasil are limited, but several studies claim that monoterpenes are present in most mango cultivars, while the presence of lactones, esters and alcohols varies among the cultivars (Pino, Mesa, Muñoz, Martí, & Marbot, 2005; Liu et al., 2013).

Based on this argument, 8 volatiles present in mango fruit which were considered the most predominant from the groups of monoterpenes, lactones, aldehydes and alcohols were selected to study the volatiles release: δ-3-carene terpinolene, menthone, ϒ- decalactone, ϒ- octalactone, decanal, nerol and geraniol.

The present chapter is focused on the optimization of the edible coating for the mango bars. Since gellan can form stable gels and allows to produce a range of different textures (Lapasin & Pricl, 1995), it has been chosen as coating material. It is well known that among the gelling agents, gellan gum presents a good 'flavour release' properties during chewing. Gibson & Sanderson, (1997) explained that the flavour release of gellan gum is due to the expulsion of fluid together with the flavour and taste compounds.

Exploratory studies by visual observation allowed the selection of a mixture of low acyl / high acyl gellan gum in the ratio L/H90/10. L gellan produces transparent and brittle gels, while H gellan produces opaque elastic gels (Philips & Williams, 2009). The formulation, L/H90/10 was considered the most suitable because it forms an elastic, but still firm coating, with a high degree of transparency.

As such, the optimization of the coating was carried out using a L/H90/10 gellan gum blend applying the Response Surface Methodology (RSM). The independent variables were the concentrations of Ca^{2+} and L/H90/10 gellan, as well as the storage time. The response variables were coating thickness, mango bars firmness, syneresis and total colour difference, after coating application on the bars. The apparent viscosity and sol-gel transition of the coating solutions were determined. These results were considered as complementary information for selecting the coating for the mango

bars. The impact of the selected coating on the volatiles release profile during nine days of storage was performed using Headspace-Solid Phase Micro Extraction-Gas Chromatography (HS-SPME-GC).

Table 4.1 Volatile compounds present in mango fruit

	Compound	*Aroma characteristics*	*Odour Activity Values (OAVs)*
Monoterpenes	δ- 3- carene	Leaf of mango tree and aroma characteristic of mango fruit[3] Sweet[1,2] and ripe[1] Lemon and resin[4]	0.7-1.4
	α - terpinolene	Lemon[4]	0.4-2.4
	Myrcene	Green mango[1,2,3] Balsamic and spice[4]	0.8-1.9
	Limonene	Lemon, orange and citric[4,5]	0.8-1.9
	α - copaene	Wood and spices[4]	
	Menthone	Fresh and ripe[4]	
Lactones	ϒ-decalactone	Herbaceous, coconut and sweet[1,2,6], Peach[3,4,5] and pineapple[3]	0.6-1.1
	ϒ-octalactone	Herbaceous[1,2], coconut[1,2,4] and sweet[1,2,6] Peach and pineapple[3]	0.4-1.1
	ϒ-undelactone	Peach[5]	
Esters	2,5–dimethyl–4–methoxyl– 3(2H) - furanone	Caramel and sweet[4]	2.8-4.8
	(E) – β - ionone	Algae, floral and raspberry[4] Lily[5]	3.4-4.6
	Ethyl butanoate	Fruity[1,2]	1.8-3.7
	Ethyl–2–Methyl propanoate	Fruity[1,]	2.3-2.9
	Methyl benzoate	Fruity[1] and floral[2] Lettuce, fennel and plum[4,5]	1.9-2.8
	(E,Z) – 2,6- nonadienal	Melon[1,2] and cucumber[2,4] Wax[4] and geranium[5]	3.0-4.5
	2,4- nonadienal	Watermelon and geranium[4,5]	
	(E) – 2 - nonenal	Melon[1,2] and cucumber[2,4]	2.9-3.9
Aldehydes	Decanal	Soap, fat, orange peel[4,5]	2.0-2.5
	Hexanal	Grass and fresh[2,4]	0.6-1.7
	α - terpineol	Oil, anise and mint[4]	0.1
	Nerol	Sweet[5], floral[6] and citric[6]	
Alcohols	Geraniol	Rose[5], geranium[4,5] and citric[6]	
	Linanool	Floral[6], lavender[4], coriander[5], floral and citrus[1,6]	0.3-1.1

[1] Pino & Mesa, 2006; [2] Pino *et al*., 2005; [3] Malundo *et al*., 1997; [4] Flavornet, avalable on: http://www.flavornet.org; [5] Fantastic Flavour, avalable on: http://fantastic-flavour.com/; [6] Chauhan *et al*., 2010.

4.3. Materials and methods

4.3.1. Materials

Mango fruits and gellan gum were purchased as described in section 2.3.1. – Chapter 2.

Calcium gluconolactate was purchased from Solegraells (Barcelona, Spain). Nerol (≥ 95 %), decanal (≥ 95 %), δ-3-carene (≥ 95 %), geraniol (≥ 98 %), ϒ- decalactone (≥ 99 %) were provided by Sigma-Aldrich (Madrid, Spain), ϒ- octalactone (≥ 96 %) from Acros Organics (Geel, Belgium), terpinolene (≥ 96 %) from Fluka (Madrid, Spain) and menthone (≥ 96 %) was purchased from Fluka Chemika (Neu-Ulm, Switzerland). Polydimethylsiloxane Solid Phase Microextraction (SPME) fibres (100µm) were purchased from Supelco (Madrid, Spain).

4.3.2. Preparation of mango bars

For the preparation of mango bars, a similar procedure was used as described in section 3.3.1. – Chapter 3. Bars with an overall gellan concentration of 1.0 %wt and low acyl/high acyl in the ratio of 25/75 were prepared (see Chapter 3, section 3.4.6 – Sensory Analysis).

4.3.3. Production and application of the coating

Coating solutions were prepared by dispersing the required amount of calcium gluconolactate for the defined Ca^{2+} concentration (Table 4.2) in distilled water at 88±2 °C. Then, gellan powder with a low acyl/high acyl gellan ratio of 90/10 at the amounts required (Table 4.2) was added under stirring at similar temperature.

The mango bars were removed from the silicone moulds. A thin wood stick was carefully introduced into the mango bars, then, the bars were dipped for 20 s into the gellan gum/calcium gluconolactate solution at 88±2 °C. The coated bars were left at room temperature (22±2 °C) for 30 minutes then stored at 5 °C for 30 min before being analysed (Figure 4.1).

Figure 4.1 Coated mango bars used for analysis.

4.3.4. Optimization of the coating formulation

Modelling and optimization of the edible coating formulation was performed using a central composite rotatable design (CCRD), as a function of three factors (variables): the calcium concentration (X_1, mM), L/H90/10 gellan concentration (X_2, %wt) and storage time (ST) (X_3, days). The variables were previously identified as having an important effect on the gellan gels characteristics and ranges were selected based on previous experiments (Garg, Kaur, Garg, & Sud, 2008) (Table 4.2). The ranges were selected based on previous experiments.

The factorial design included 20 experiments:

1. Eight data-points (extremes), which represent the vertices of a *p*-dimensional cube, at a distance of $p^{1/2}$ from the origin of the coded system of reference at levels (−1) and (+1).
2. Six axial points outside of the factorial matrix, but inside the experimental domain, at a distance equal to $2^{p/4}$ from the origin (star-points). These levels correspond to −1.6818 and 1.6818.
3. A third set composed by the replicates of the points at the origin of the reference system (central-points), coded as (0, 0).

The response variables studied were: coating thickness (Y_1; mm), mango bars firmness (Y_2; *N*), syneresis (Y_3; *%*), and mango bars total colour difference (ΔE*) (Y_4) (Table 4.2). The experimental data was fitted to the model in Equation 4.1 (Box & Behnken, 1960).

$$= b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{23}X_2X_3 + b_{11}X_1^2 + b_{22}X_2^2 + b_{33}X_3^2 \qquad \text{Eq. 4.1}$$

where Y_i (i = 1, 2, 3 or 4) are the response variables; b_0 is the intercept; b_1-b_{33} are regression coefficients computed from the observed experimental values of Y; and X_1-X_3 are the coded levels of independent variables.

Table 4.2

Decoded experimental design matrix used (CCDR) as a function of independent variables: (Ca^{2+}) calcium concentration, gellan (L/H90/10) concentration, (ST) storage time; and results (mean ± SD) of coating thickness and mango bar firmness, syneresis and colour difference (ΔE*)

Run	Independent variables			Dependent variables			
	Factors			Responses			
	Ca^{2+} (mM) X_1	Gellan (%) X_2	ST (days) X_3	Coating thickness (mm) Y_1	Firmness (N) Y_2	Syneresis (%) Y_3	ΔE* Y_4
1	2.98	0.45	3	0.13	0.93	0.41	3.67
2	2.98	0.45	8	0.14	1.16	1.57	1.82
3	2.98	1.20	3	0.73	1.69	0.57	2.75
4	2.98	1.20	8	0.78	1.96	1.10	2.01
5	7.98	0.45	3	0.11	0.74	0.80	2.49
6	7.98	0.45	8	0.12	1.18	1.51	3.70
7	7.98	1.20	3	0.76	0.88	0.74	2.04
8	7.98	1.20	8	0.91	1.37	0.78	2.30
9	0.00	0.75	3	0.20	1.36	1.10	2.46
10	10.00	0.75	3	0.24	1.33	0.97	0.00
11	0.00	0.00	3	0.00	1.20	1.64	1.66
12	5.00	1.50	5	1.12	1.50	0.68	2.63
13	5.00	0.75	0	0.23	0.88	0.00	3.05
14	5.00	0.75	10	0.28	0.90	1.56	1.27
15	5.00 (CP)	0.75	5	0.17	0.85	1.05	4.49
16	5.00 (CP)	0.75	5	0.19	0.87	0.96	4.46
17	5.00 (CP)	0.75	5	0.20	0.97	0.72	4.39
18	5.00 (CP)	0.75	5	0.18	1.13	0.99	4.71
19	5.00 (CP)	0.75	5	0.16	0.82	1.11	4.50
20	5.00 (CP)	0.75	5	0.19	0.86	1.01	4.49

4.3.5. Rheology of the coating solutions

The apparent viscosity and sol-gel transition of the gellan gum solutions of the experimental design (Table 4.2) were characterized using a Thermo Scientific HAAKE MARS rheometer, equipped with a Peltier heating system. The gelling temperatures of L gellan and H gellan are around 80 ºC and 30 ºC (Philips & Williams, 2009). For this reason, all the rheological measurements were carried out at

90 ºC to prevent gel formation. The shearing surfaces in contact with air were covered with paraffin oil in order to prevent water evaporation.

Flow curves were obtained using a cone and plate geometry (angle 2º, diameter 3.5 cm). The heated coating solution (90±2 °C) was placed on the preheated geometry (90°C) and subjected to a shear rate ($\dot{\gamma}$) range between 0.01 and 1000 s^{-1}. Apparent viscosity (η) was recorded as a function of shear rate. The gelling temperatures of the coating solutions were studied through small amplitude oscillatory shear measurements using parallel serrated plates with a diameter of 2 cm, maintaining a constant gap of 1.0 mm. The heated coating solution (90±2 °C) was loaded to the preheated plates (90 ºC) and subjected to an oscillatory temperature sweep from 90 ºC to 20 ºC at a rate of 1 ºC/min, f = 0.1 *Hz* and τ = 0.1 *Pa*. The constant stress was chosen based on stress sweeps at constant frequency (f = 0.1 *Hz*), in order to assure conditions within the linear viscoelastic region. All measurements were carried out in duplicate.

4.3.6. Measurement of the experimental design response variables

Coating thickness

The thickness (*mm*) of the coating formulations was assessed using a digital micrometer (Model CD-6"BS, Mitutoyo Co., Japan) with an accuracy of ± 0.001 mm. These measurements were carried directly on the coated mango bars to avoid mechanical damage that could be caused by removing the coating from the mango bars. Four measurements in different points on each testing sample were carried out.

Firmness

Firmness (*N*) of the coated mango bars was measured with a Texture Analyzer model TA.TX Plus (Stable Micro Systems, Ltd., UK) equipped with a 50 N load cell. The mango bars with dimensions of: W x H x L =27 x 10 x 50 mm were punctured up to 50 % of the initial height using a cylindrical probe with a flat base of 8 mm diameter, operating at a crosshead speed of 0.8 mm s^{-1}. Firmness was measured as the maximum force recorded in the force–time curve during compression (Fiszman & Salvador, 1999; Bourne, 2002).

Syneresis

Syneresis was performed as described in section 3.3.2. – Chapter 3. At regular intervals, (Table 4.2) the condensed water from the cell walls and samples surfaces was removed with tissue paper and the samples were weighed again. Reported values are averages of four independent measurements.

Total colour difference

The colour alterations on the mango bars were determined with a colorimeter (Minolta CR 300, Switzerland). CIELAB, L* (lightness), a* (red-green) and b* (yellow-blue) colour space was applied to perform colour measurements and the total colour difference (ΔE*) was calculated according to the following equation:

$$\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \quad \text{Eq. 4.2}$$

where, L* value expresses the lightness or luminosity of colour, which varies from 0 (absolute black) to 100 (absolute white); a* is associated with changes in redness-greenness (positive a* is red and negative a* is green); and b* is associated with changes in yellowness-blueness (positive b* is yellow and negative b* is blue). $\Delta L^* = (L_1^* - L_0^*)$; $\Delta a^* = (a_1^* - a_0^*)$; $\Delta b^* = (b_1^* - b_0^*)$. Subscript '0' refers to the reference (uncoated sample) and subscript '1' refers to each sample being analysed.

4.3.7. Evaluation of volatiles release by gas chromatography

Sample preparation

Samples were prepared by dispersing the gellan powder into mango puree as described in section 3.3.2. – Chapter 3. A mass of 10 g of the heated mango puree/gellan mixture (90±2 ºC) was transferred into a 40 mL glass vial (suitable for headspace gas chromatography analysis) and allowed to set at room temperature for 15 min. During this time, the vials remained opened to minimize condensation of water vapour, after that they were sealed and cooled down to 5 ºC for 15 min. Meanwhile, the coating solution was prepared using the method previously described in section 4.3.3. The application of the coating to the texturized mango puree consisted on the addition of 1.5 mL of heated coating solution (1.0 %wt L/H90/10 and 6 mM calcium gluconolactate value - selected from the experimental design) to the surface exposed to headspace inside the vial.

All vials were stored at refrigerated conditions during 9 days. At regular time intervals, two vials per type of sample (duplicate) - coated and uncoated texturized mango puree - were analysed with the procedure described in the next item, and discharged.

Instrumental analysis of HS-SPME-GC

The analysis of the volatile compounds was performed by Headspace-Solid Phase Micro Extraction-Gas Chromatography (HS-SPME-GC). For the extraction of volatile compounds, the vials with the samples were heated at 60 ºC in a water bath for 20 min, afterwards, a polydimethylsiloxane SPME fibre was inserted into the headspace of the vial and exposed for 30 min. The fibre was then removed from the headspace and inserted into the injector at 250 °C during 5 min for volatiles

desorption. According to this procedure, the higher the peak areas obtained, the higher the amount of volatiles. Two replicates were carried out for each sample.

The analysis were performed using a Varian 450-GC gas chromatograph equipped with a flame ionisation detector and a capillary column (Agilent Technologies, Diegem, Belgium) with the dimensions: diameter x length x film thickness = 0.25 mm x 30 m x 0.25 µm. Ultra high purity helium was used as a carrier gas at a constant flow rate of 1 mL min^{-1}. The column oven temperature was held at 40 ºC for 2.5 min, ramped at 5 ºC m^{-1} until 200 ºC, held for 5 min, then ramped from 10 ºC m^{-1} to 240 ºC and held for 15 min. The conditions employed are similar to those found in the literature regarding the analysis of the mango fruit volatile compounds (Malundo, Baldwin, Moshonas, Baker, & Shewfelt, 1997; Pandit et al., 2009; Pino & Mesa, 2006; Shang et al., 2002) and other tropical fruits (Cardeal, Guimarães, & Parreira, 2005; Ibanez et al., 1998). Eight aroma compounds were identified by the retention time of the respective standard: δ-3 carene, terpinolene, menthone, ϒ- octalactone, ϒ-decalactone, decanal, geraniol and nerol.

4.3.8. Cryo Scanning Electron Microscopy (Cryo-SEM)

Cryo-scanning electron microscopy was performed as described in section 3.3.5 – Chapter 3.

4.3.9. Statistical analysis

Multiple linear regression analysis was used to fit the model (Equation 4.1) to the experimental data of CCRD. The linear and quadratic effects were calculated and their significance was evaluated by analysis of variance (*p*-value and *F*-value at 95 % confidence level). The fit of the models was evaluated by the determination coefficients (R^2) and adjusted R^2 (R_{adj}^2). A 3D surface, described by a second order polynomial equation was fitted to each set of experimental data points. First and second order coefficients were generated by regression analysis. The response surfaces are represented in 3D surfaces as a function of two independent factors keeping the third factor constant at the value of the central point (storage time = 5 days; Ca^{2+} concentration = 4.9 mM; gellan concentration = 0.78 %wt)

Data of volatile compounds of uncoated and coated mango bars with the selected coated formulation, during storage period were subjected to principal component analysis (PCA). All the statistical analysis were carried out using the software STATISTICA version 6.0 (2001) (StatSoft Inc., Tulsa, OK, USA).

4.4. Results and discussion

4.4.1. Rheology of coating solutions

Steady shear measurements

The apparent viscosity of 0.45, 0.75, 1.2 and 1.5 % wt gellan solutions (L/H90/10) with different Ca^{2+} concentrations is shown in Figure 4.2. All solutions displayed a non-Newtonian shear thinning behaviour. In addition, a rise in the apparent viscosity was observed as the Ca^{2+} concentration increased for each gellan concentration studied. The gelation process of L gellan is known to be highly dependent on the cations concentration. The cations promotes helices aggregation by site binding between pairs of carboxylate groups within the polymer chain leading to the formation of a gel network upon cooling (Morris, Nishinari, & Rinaudo, 2012). Considering the high proportion of L gellan in the formulation L/H 90/10, a higher Ca^{2+} concentration was expected to promote a higher number of electrostatic interactions between pairs of carboxylate groups, giving rise to a more viscous solution before the gelation process takes place.

When gellan/Ca^{2+} concentrations increased up to 1.50/7.98 no marked increase of the viscosity was noticed at lower shear-rates. As the shear-rate increases to 1000 s^{-1} the difference in viscosity between the solutions was reduced (Figure 4.4c). However, all the solutions in Figure 4.2 became less viscous at higher shear-rate.

In order to describe the flow behaviour of the solutions, several rheological models, e.g. Cross, power-law and Oswald de Waele models, depending on the flow curve shape, were fitted to experimental data. However, these fittings were not successful.

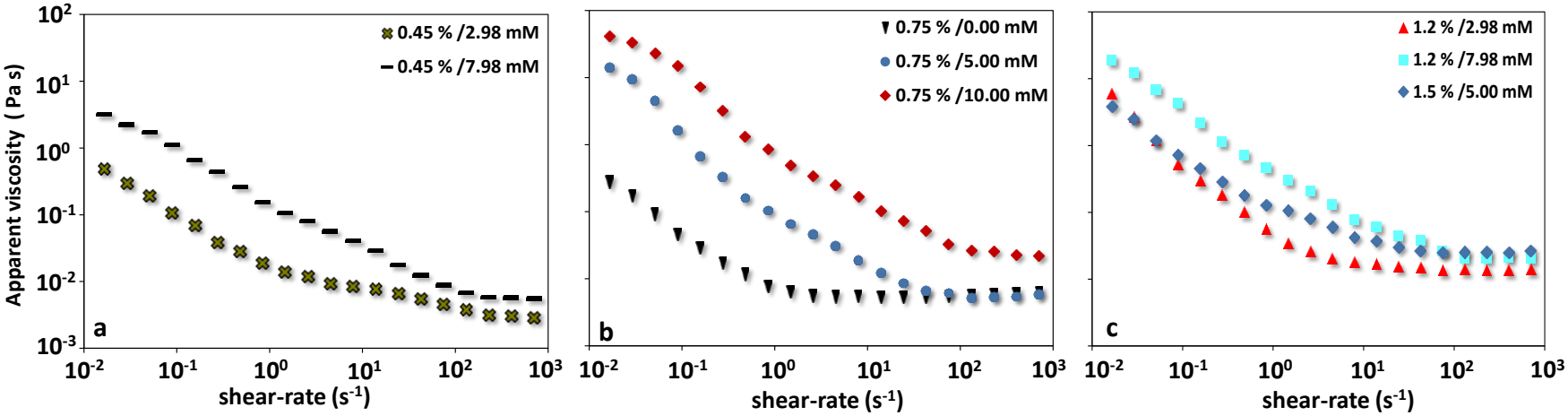

Figure 4.2 Flow curves of the coating solutions at 90°C for various concentrations of L/H90/10 gellan (% wt)/Ca^{2+} (mM).

Dynamic oscillatory measurements

Figure 4.3 shows the effect of L/H90/10 gellan concentration and Ca^{2+} concentration on the variation of the storage (G') and loss (G") moduli with temperature. In Figure 4.3a is presented the temperature sweep for the formulation with the lowest L/H90/10 gellan concentration (0.45%) for Ca^{2+} contents of 2.98 and 7.98mM. It can be seen that as the temperature decreases from 90 to 80 °C, G" is larger than G', denoting a predominant viscous behaviour. A cross-over of the moduli occurs at the temperatures around 77 °C for 0.45 %/7.98 mM and 73 °C for 0.45 %/2.98 mM which marks the onset of the structure development defined as the sol-gel transition (T_{sg}). Afterwards, the dynamic moduli continue to rise slowly with decreasing temperature until a marked increase without a cross-over of the moduli at around 38 °C for 0.45 %/7.98 mM and 29 °C for sample 0.45 %/2.98 mM was noticed.

As the L/H90/10 and Ca^{2+} concentrations increases, the values of the moduli tend to increase over the temperature sweep (Figure 4.3b). Figure 4.3b show a pattern similar to the one in Figure 4.3a with a cross-over of the moduli at high temperatures (around 75 °C) and a rise of the moduli without crossover at low temperatures (around 35 °C). Kasapis et al., (1999) and Matsukawa & Watanabe, (2007), also observed two regions of steep increase in dynamic moduli for L/H gellan blends. The first increase occurs at high temperatures coincident with the T_{sg} of H acyl gellan and the second corresponding with the transition of the L acyl at much lower temperatures. This is in line with the studies of Differential Scanning Calorimetry of the L/H gellan mixtures that indicate the presence of two separate conformational transitions at temperatures that are characteristic of individual components (Morris, Gothard, Hember, Manning, & Robinson, 1996).

The samples 1.2 %/2.98 mM and 0.75 %/5.00 mM present closer T_{sg} values 79 °C and 77 °C respectively. For the sample 0.75 %/0.0 mM, the sol-gel transition occurred at lower temperature (72 °C). These values show that samples with a lower Ca^{2+} concentration reach the gel state at lower temperatures (Figure 4.3b). In addition, the sample in which no Ca^{2+} was added, shows a much lower rise of dynamic moduli at temperature around 25 °C, identified as that corresponding to L acyl gellan gel formation. This fact may be attributed to the high dependence of this type of gellan on Ca^{2+} concentration for gel formation, resulting in a weaker L acyl gellan gel phase within the jellified sample when a lower amount of Ca^{2+} is present.

In Figure 4.3c are grouped the samples containing the higher Ca^{2+} concentrations tested. Independently of the L/H gellan concentration, they exhibit a G' larger than G" in the whole temperature range studied, including at temperatures near 90 °C. This last fact is perceived when comparing the samples 0.75 %/5.00 mM (Figure 4.2b) and 1.5 %/5.00 mM (Figure 4.2c).

These results indicate a more elastic system that may be a consequence of a higher number of electrostatic interactions between L gellan molecules promoted by the high cation content. This is in accordance with the higher apparent viscosity of the same formulations (Figure 4.4c). Still, a substantial increase of both moduli at temperatures ranged between 43 and 29 °C is observed, which indicate the gel formation by L gellan.

Overall, the T_{sg} of the L/H90/10 mixtures occurs at higher temperatures as the Ca^{2+} and polymer concentrations increased. With increasing the amount of polymer and Ca^{2+}concentration a more extensive helices aggregation occurs (involving the H gellan portion of the L/H blend) and thus the sol-gel transition takes place at higher temperatures.

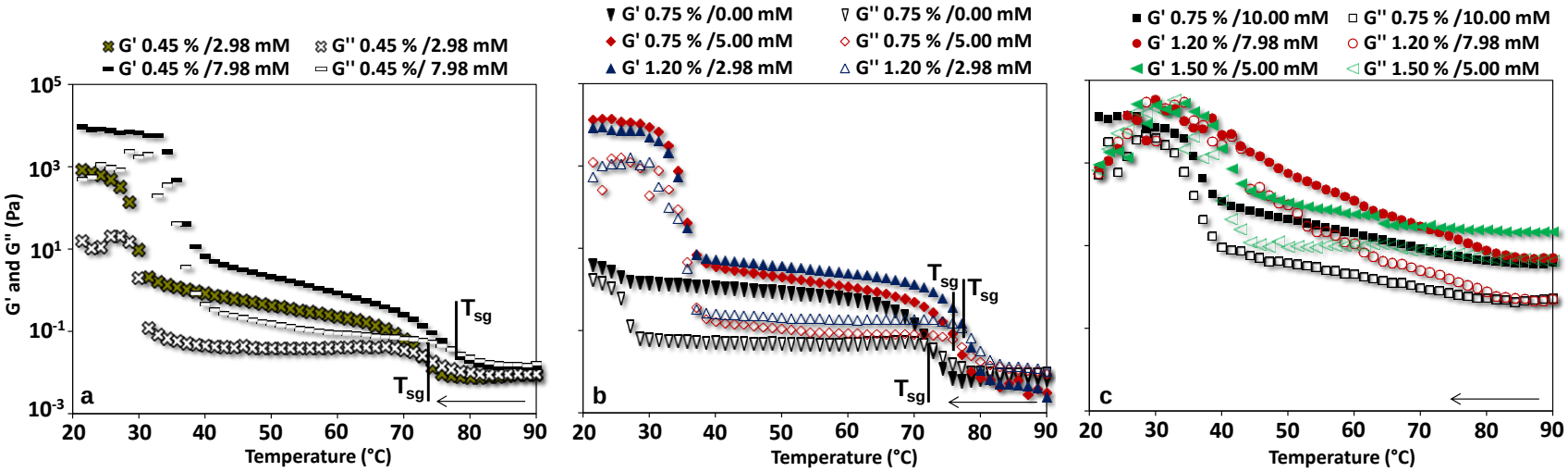

Figure 4.3 Variation of storage moduli (G') and loss moduli (G") on cooling for coating solutions at various L/H90/10 gellan and Ca^{2+} concentrations.

4.4.2. Optimisation of coating formulation by Response Surface Methodology

The linear and quadratic effects of the factors, as well as their linear interactions, (for coating thickness, mango bar syneresis and ΔE*) are presented in Table 4.3 The high values of both R^2 and R_{adj}^2 indicated a good fit of the models to experimental results.

Coating thickness

According to the equation from Table 4.3 – L/H90/10 gellan concentration was the single factor showing a significant positive effect, linear and quadratic, on the coatings thickness ($p < 0.05$). It remained constant (<0.2 mm) until 0.4 %wt gellan, then increased progressively with the gellan concentration up to a maximum of 1.2 mm. (Figure 4.4a). The linear and quadratic terms of storage time were non-significant ($p > 0.05$), showing that the storage time did not influence the coating thickness. This fact may also be observed in Figure 4.4b, in which the coating thickness is maintained constant when increasing the storage time. The linear, quadratic and interaction terms of Ca^{2+} showed to be non-significant ($p > 0.05$) evidencing that this variable had no effect on mango bars coating thickness.

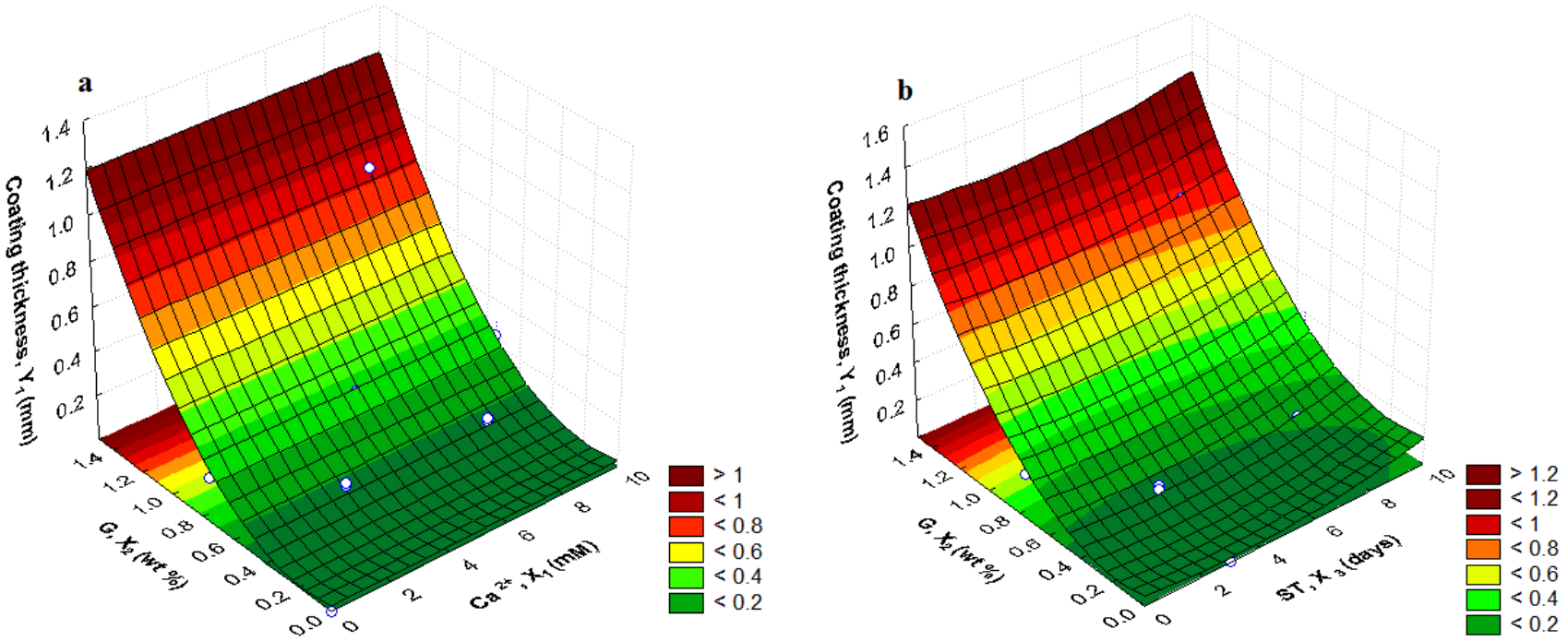

Figure 4.4 Response surface plots for the coating thickness (Y1) of the mango bars as a function of (a) gellan concentration (X_2) and Ca^{2+} concentration (X_1), (storage time (X_3) = 5 days); (b) gellan concentration (X_2) and storage time (X_3), (Ca^{2+} concentration (X_1) = 4.9 mM).

Table 4.3

Model equations describing the response surfaces fitted to the CCRD experimental data points. Coating thickness, coated bars syneresis and coated bars ΔE* as a function of the gellan concentration, Ca^{2+} concentration and storage time and respective R^2 and R^2_{adj}

Dependent variable	Equation	R^2	R^2_{adj}
Coating thickness (Y_1)	$0.30 + 0.04Ca^{2+} + 0.65\ G^* + 0.19G^{2*} + 0.05ST + 0.03ST^{2+}$	0.976	0.966
Coated bars syneresis (Y_3)	$0.76 + 0.30(Ca^{2+})^{2*} - 0.35G^* + 0.16G^2 + 0.64ST^* + 0.02Ca^{2+}G^* - 0.12Ca^{2+}ST - 0.28\ GST^*$	0.94	0.86
Coated bars ΔE* (Y_4)	$4.34 - 0.87\ (Ca^{2+})^{2*} - 0.65G^* - 1.44G^{2*} - 0.82ST^{2*} + 0.72\ Ca^{2+}\ ST^*$	0.938	0.89

*Significant at $p < 0.05$, Ca^{2+}– concentration of calcium (mM), G – concentration of L/H90/10 gellan (% wt); ST – storage time; R^2 – quadratic correlation Coefficient; R_{adj}^2 – adjusted quadratic correlation coefficient.

Syneresis of the coated mango bars

Polymer concentration, gel strength and compression forces are factors that can affect syneresis (Stephen, Phillips, & Williams, 2010). The syneresis was relatively low in all conditions tested; however, the linear effect of gellan concentration and storage time, and the quadratic effect of Ca^{2+} concentration, affect significantly mango bars syneresis ($p < 0.05$). Positive effect of Ca^{2+} concentration at quadratic level was reflected by the concave response surface (Fig. 4.5a).

Response surface plots show that a minimum value of syneresis is obtained for gellan and Ca^{2+} concentrations in the ranges of 0.8 to 1.2 % and 3.0 to 8.0 mM, respectively (Figure 4.5a). In addition, syneresis increased with storage time for the all conditions of gellan and Ca^{2+} concentration studied (Figures 4.5b and 4.5c).

Total colour difference in coated mango bars

Figure 4.8 describes the effect of all variables on total colour difference of the mango bars. The difference in colour was significantly ($p < 0.05$) influenced by the linear and quadratic effect of L/H90/10 gellan concentration, negative quadratic effect of Ca^{2+}concentration and storage time and positive interaction effect between concentrations of Ca^{2+} and storage time (Table 4.3). When storage time was fixed at a value of 5 (days), L/H90/10 gellan concentration and Ca^{2+} concentration displayed a quadratic effect on total colour difference response (Figure 4.6a). The same effect happened when Ca^{2+} concentration was fixed at a value of 4.9 mM (Figure 4.6b). In both cases, tested gellan concentrations (0.6 to 1 %) and Ca^{2+} concentration (4 to 7 mM) produced bars with maximum values of ΔE^* around 3.

However, the colour alterations were quite low during the entire storage time ($\Delta E^* < 5$), (Patras, Brunton, Da Pieve, Butler, 2009) indicating a coating with a rather good transparency even at higher concentrations of Ca^{2+} and/or L/H90/10 gellan.

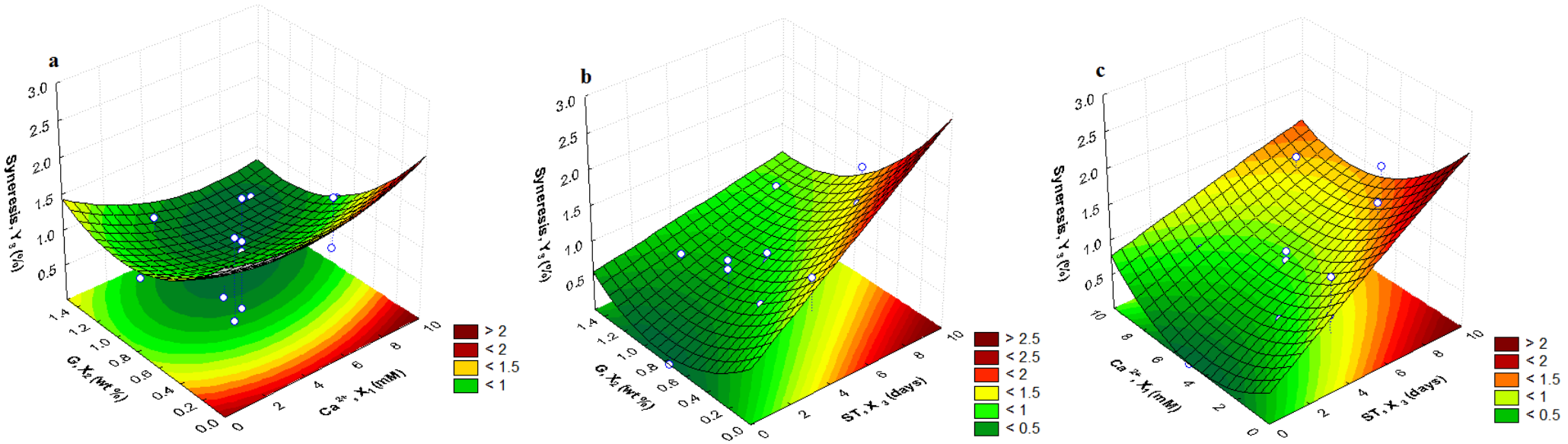

Figure 4.5 Response surface plots of mango bars syneresis (Y_3) as a function of (a) gellan concentration (X_2) and Ca^{2+} concentration (X_1), (storage time (X_3) = 5 days); (b) gellan concentration (X_2) and storage time (X_3), (Ca^{2+} concentration (X_1) = 4.9 mM); (c) Ca^{2+} concentration (X_1) and storage time (X_3), (gellan concentration (X_2) = 0.78 %wt).

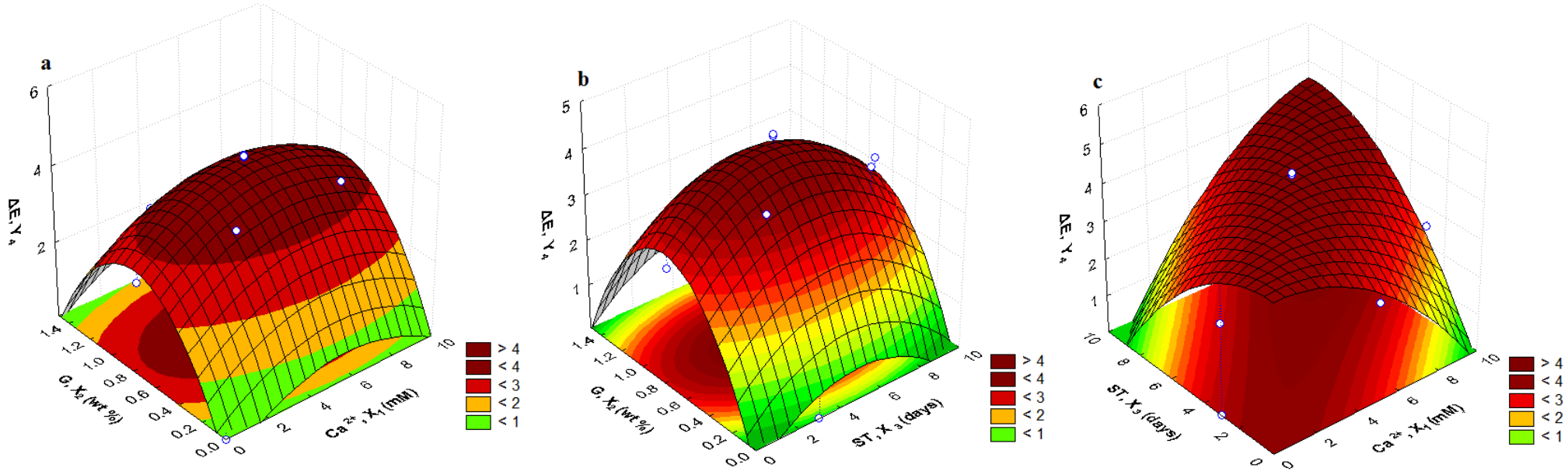

Figure 4.6 Response surface plots of total colour difference (Y_4) of the mango bars as a function of (a) gellan concentration (X_2) and Ca^{2+} concentration (X_1), (storage time (X_3) = 5 days); (b) gellan concentration (X_2) and storage time (X_3), (Ca^{2+} concentration (X_1) = 4.9 mM); (c) storage time (X_3) and Ca^{2+} concentration (X_1), (gellan concentration (X_2) = 0.78 %wt).

Firmness of the coated mango bars

The fit of the quadratic model to experimental data of bars firmness was not considered satisfactory. Though, the response surface presented in Figure 4.7 suggests an increase of firmness with increasing gellan concentration for Ca^{2+} concentrations below 6mM. Furthermore, for high contents of both calcium and gellan, the surface suggests a lower firmness than expected. This fact may be related to the formation of a more brittle structure when both calcium and gellan are present in higher amounts.

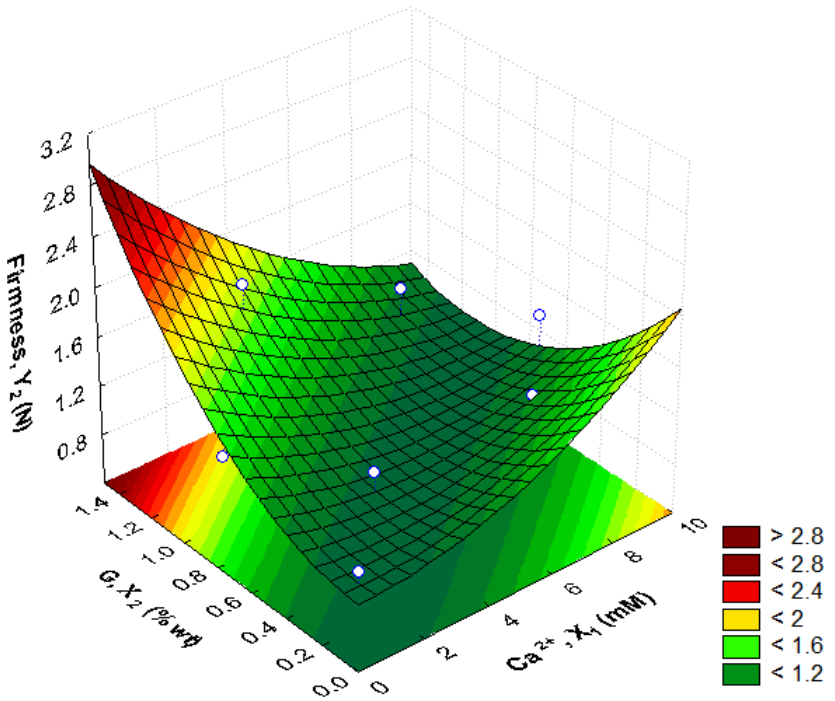

Figure 4.7 Response surface plot of the firmness (Y_2) of the mango bars as a function of gellan concentration (X_2) and Ca^{2+} concentration (X_1), (Storage time (X_3) = 5 days).

The results of the experimental design indicate that above 1.0 %wt gellan, the thickness of the coating reached 1.2 mm, which may affect the appearance of the final product. Regarding syneresis, the lower values (0.6-0.8 %) are predicted for the coatings formulations with concentration of L/H90/10 gellan and added Ca^{2+} in the ranges 1.0-1.2 %wt and 4-6 mM, respectively. Total colour differences along the storage time were influenced by both L/H90/10 gellan and Ca^{2+} concentrations. However, for gellan concentration between 1.0-1.2 %wt and Ca^{2+} concentration between 4-6 mM, the model predicted a difference in colour around 3 in the last day of storage, indicating a practically imperceptible colour difference by the human eye.

Taken into account the evaluation described above, it was considered that 1.0 wt % L/H90/10 with addition of 6 mM Ca^{2+} could represent the optimal coating formulation for the mango bars.

4.4.3. Effect of the selected coating on volatiles release

The release of eight volatiles compounds from coated and uncoated mango bars stored at refrigerated conditions (4 °C) was analysed over time by HS-SPME-GC (Figure 4.8). Appendix E displays the chromatograms obtained from mango bars (Figure E1) and coated mango bars (Figure E2).

During the entire storage period of the uncoated mango bars, δ-3-carene and terpinolene were present in the headspace in a significant higher concentration when compared with the other volatile compounds (Figure 4.8a). Beaulieu & Lea, (2003); Malundo et al., (1997) reported the same behaviour for mango fruit, where terpenes, particularly δ-3-carene dominated the volatile profiles. δ-3-carene and terpinolene presented higher peak areas until the third day of storage, followed by an abrupt decrease in the sixth day, maintain afterwards almost the same value until the last day of refrigerated storage. Menthone, decanal, ϒ-octalactone and geraniol presented smaller peak areas and a similar behaviour during the entire storage time. Nerol and ϒ-decalactone have shown low values of peak areas, in the range of the former compounds, maintaining constant peak areas during the entire storage period (Figure 4.8a).

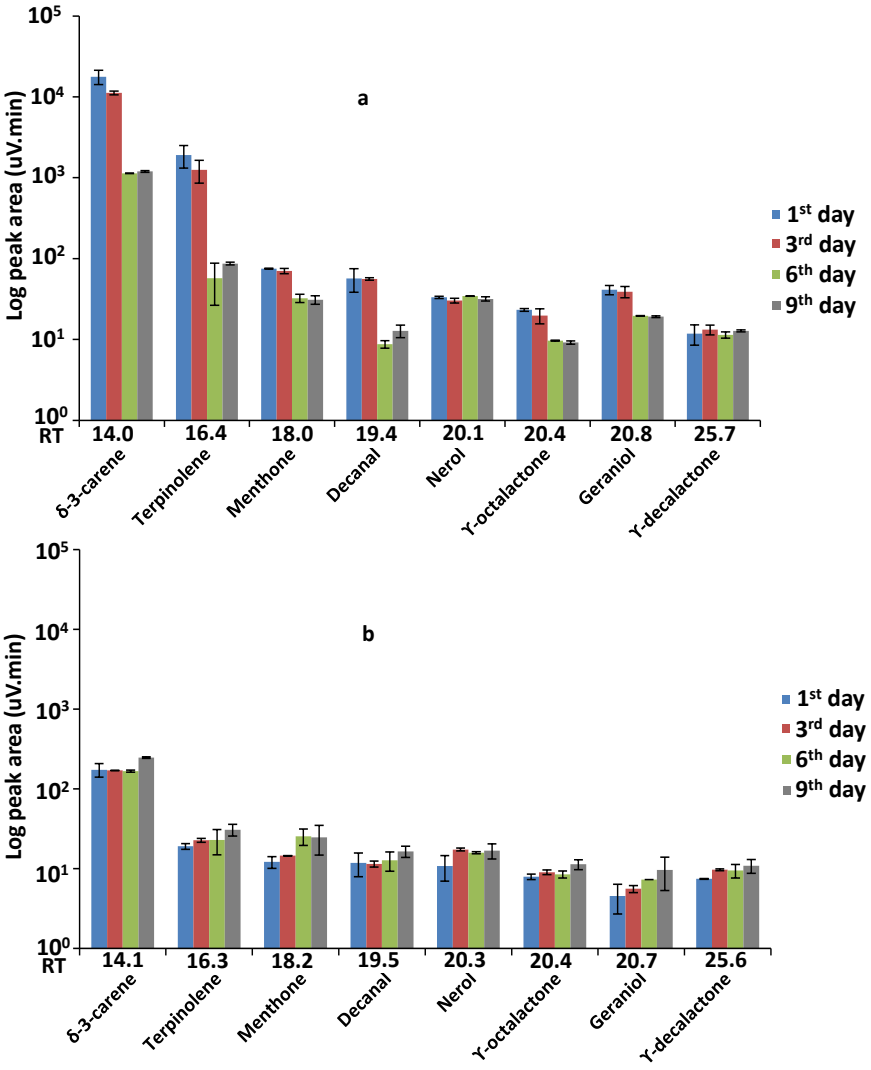

Figure 4.8 Mean values (± standard deviation) and retention times (RT) of volatile compounds during 9 days of storage for (a) mango bars and (b) coated mango bars.

In the presence of coating, lower peak areas are observed for all the volatiles being this fact particularly significant for δ-3-carene and terpinolene (Figure 4.8b). These results may be a consequence of a lower compounds release to the headspace due to a barrier effect created by the coating. The values of the peak areas are relatively stable during the entire storage time.

To obtain an overview of the effect of the coating on the release of volatile compounds of mango bars, a multivariate analysis (Principal Component Analysis) was performed considering the mean values of peak areas of the eight volatile compounds. The two first principal components (PC1 and PC2) accounted for 90.6 % of the total variance (Figure 4.9). A good discrimination is perceived between coated mango bars (cMB) and uncoated mango bars (MB) during the entire storage time, indicating the coating effect on volatiles retention.

PC1 is explained by the majority of the volatile compounds (decanal, geraniol, nerol, ϒ-octalactone and ϒ-decalactone). Uncoated mango bars in the last days of storage form a cluster at an Euclidian distance of 4 related with those compounds.

Uncoated mango bars (MB) in the first and third day of storage are grouped on the positive side of PC2, explained by δ-3-carene and terpinolene, indicating a higher release of these two volatiles in the first days of storage.

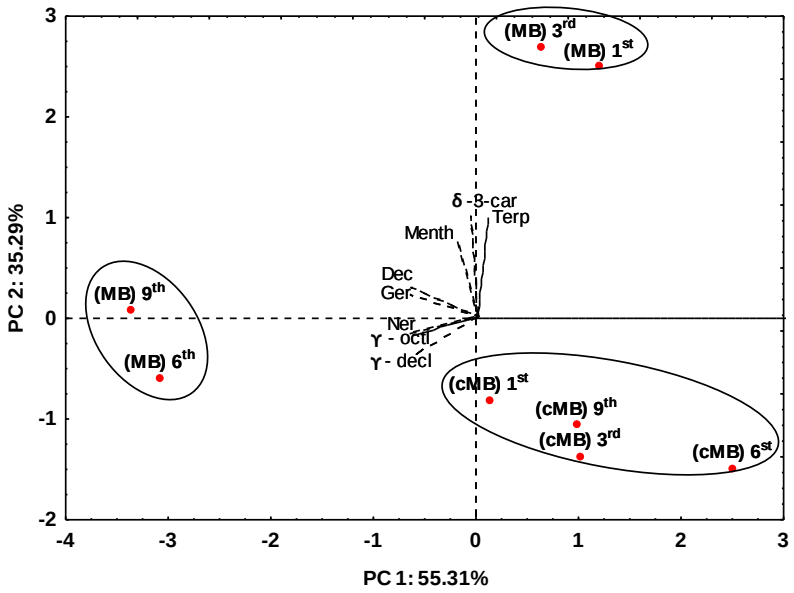

Figure 4.9 PCA biplots of variable coordinates of volatile compounds during 9 days of storage (5 °C) for mango bar (MB) and coated mango bars (cMB).
(MB) 1st, 3rd, 6th and 9th: the total peak area of the mango bar in the first, third, sixth and ninth day of storage; (cMB) 1st, 3rd, 6th and 9th: the total peak area of the coated mango bar in the first, third, sixth and ninth day of storage; δ-3-car - δ-3-carene, Terp – Terpinolene, Dec – Decanal, Ger – Geraniol, Menth – Menthone, ϒ-octl - ϒ-octalactone, Ner – Nerol, ϒ-decl - ϒ-decalactone.

4.4.4. Cryo-SEM observations

Considerable differences of the Cryo-SEM micrographs of L/H90/10 gellan gels were noticed upon addition of Ca^{2+}. Figure 4.10A shows the structure of coatings from low acyl gellan without calcium addition, displaying a structure with larger pores with an arrangement similar to a honeycomb structure. The addition of 6 mM Ca^{2+} to the L/H90/10 gellan (Figure 4.10B) induced the establishment of a large degree of inter-chain aggregation with the formation of a highly interconnected structure with smaller pores.

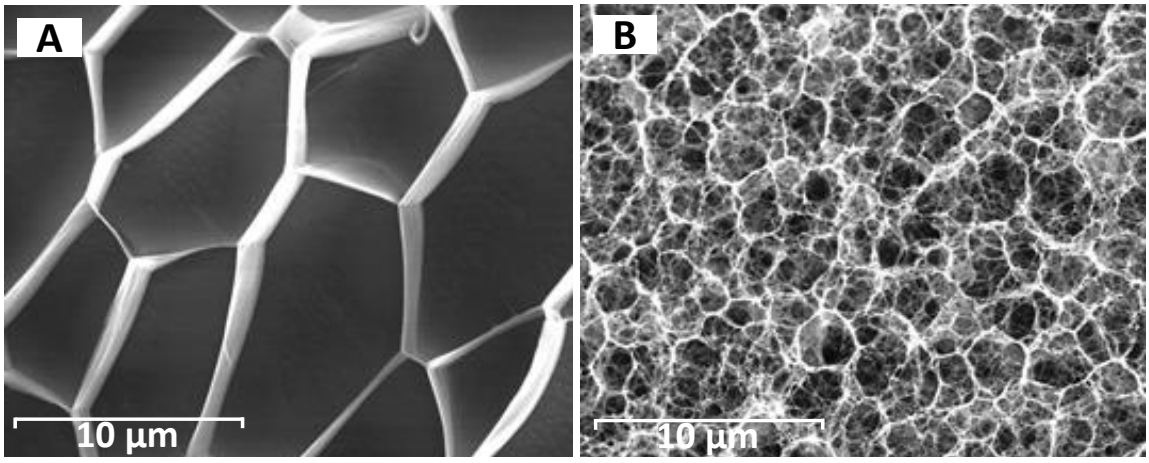

Figure 4.10 CryoSEM images for 1 %wt gellan gels at a scale of 10 µm. (A) L/H gellan gum at the ratio of 90/10 without addition of Ca^{2+}, (B) gellan gum at a ratio of L/H 90/10 with 6 mM Ca^{2+}.

4.5. Conclusions

In the process of optimisation of an edible coating based on L/H90/10 gellan gum for ready-to-eat mango bars, the rheological measurements of all the coating formulations showed a strong shear thinning behaviour, where the apparent viscosity was influenced by both gellan and Ca^{2+} concentrations. Upon dynamic oscillatory temperature sweeps, they displayed two regions of steep increase in dynamic moduli: the first, at high temperatures, coincident with the sol-gel transition due to the jellification of the H gellan fraction; and the second, at low temperatures, related with a reinforcement of the structure, due to jellification of the L gellan fraction.

Response surface methodology helped to understand the importance of coating on mango bars and on the selection of the coating optimal formulation. Gellan concentration is the independent variable that influences most the thickness of the coating, an aspect that is important for the appearance of the product.

Concentrations of both L/H90/10 gellan and Ca^{2+} in the coating formulation have a high influence on syneresis. Clearly, the coating with concentrations of L/H90/10 gellan in the range of 0.8 to 1.2 % and concentrations of Ca^{2+} between 3.0 to 8.0 mM, lowered the syneresis, which is important since syneresis directly affects the quality of the product throughout moisture.

Values of total colour change (ΔE^*) revealed the colour differences among the coating formulations depends mainly on the concentrations of gellan and Ca^{2+}. However, the colour alterations were quite low during the entire storage time ($\Delta E^* < 5$), indicating good colour stability.

Considering the model predictions, 1.0 %wt L/H90/10 with addition of 6 mM Ca^{2+} could represent the optimal coating formulation for the mango bars. Multivariate analysis showed that the

presence of the selected coating constitutes a barrier that reduces the amount of volatiles released during storage time.

The results suggest that the presence of coating improves the appearance, reduces syneresis and the release of volatiles during storage time, in consequence might be an important tool to increase the organoleptic characteristics and commercial value of the final product.

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Chapter 5

Effect of thermal and high hydrostatic pressure treatments on mango bars shelf-life under refrigeration

5.1. Summary

Ready to eat mango bars, developed in the previous chapter, were packaged using a heat-sealable multilayer material (PET/PE aluminium film). Afterwards they were subjected to two types of conservation treatments: thermal treatments (TT) (80 and 90 °C for 120 and 300 seconds) and high hydrostatic pressure (HHP) (500 MPa for 120 and 240 seconds), in order to compare their impact on flavour release and microbial stability of the mango bars. It was observed a quite similar effect of both thermal and non-thermal treatments on the referred bars characteristics. As such, heat treatment at 80ºC for 120 seconds was selected as it is less expensive than HHP, and it is the one, from the heat treatments tested, that is carried out at lower temperature and during a lower period of time. Several parameters such as, hardness, cohesiveness, springiness, total colour difference, browning index, sensory quality and microbial growth (determination of aerobic mesophillic bacteria, yeasts and mould and *Listeria monocytogenes* pathogen bacteria) were monitored over time.

Results allow concluding that the thermal treatment is important to provide a longer shelf-life. Bars have shown a good stability during refrigerated storage, as the monitored parameters did not change significantly. The shelf life of the developed product is estimated to be around 17 days.

5.2. Introduction

An active promotion of fruit as a basic component of a healthy diet resulted recently in the development of a range of ready-to-eat fresh fruit products and this became an area of interest in the food industry. In fact, consumption of fruits and their products is associated with a decreased risk of degenerative diseases such as cancer and coronary heart disease, due to health promoting phytochemicals such as carotenoids, flavonoids, phenolic compounds and vitamins (Hansen, Purup, & Christensen, 2003).

From a quality point of view, it is desirable to preserve the characteristics of the fruit products. The consumers consider their fresh-like appearance, taste and flavour, in addition to their convenience, the most appealing attributes for these products. Microbial spoilage, discoloration or browning, textural changes and development of off-flavor or off-odour are among the limitations of the shelf-life of the fruit products.

Food preservation technologies

Food industry needs to ensure the safety of the products while maintaining the quality. The loss of compounds responsible for aroma during food processing operations is one of the major problems in food industry. The control of microbial access and growth in food products is important to ensure consumers' health and well-being and to minimise loss of food through spoilage.

Thermal treatment (TT)

Thermal treatment is the most widely used process technology in the food industry which ensures microbiological safety and enzymes inactivation of the products, as it is less expensive and more accessible than the other processing treatments. Several studies on thermally treated fruit products such as guava puree (Yen & Lin, 1996), tomato and carrot purees (Patras, Brunton, Butler, & Downey, 2009), melon juice (Chen et al., 2009), fruit smoothies (Keenan, Brunton, Ronan, Butler, Tiwari, & Patras, 2010) and tamarillo fruit (Mertz, Brat, Caris-Veyrat, & Gunata, 2010) reported loss of nutritional quality, thus reducing the beneficial health effects as well as sensory quality. Nowadays, new technologies are being explored with lower impact on overall quality than traditional thermal processes.

High hydrostatic pressure (HHP)

High hydrostatic pressure, also known as ultra-high pressure (UHP), is an established non thermal food processing and preservation technique with reduced effects on nutritional and quality parameters compared to conventional thermal processing (Barbosa-Cánovas et al., 2005). At pressures higher than 300 MPa a significant inactivation of vegetative microbial forms is observed even at ambient temperatures within treatment times of several minutes. By increasing the pressure to 600 MPa (at

present the technical limit of industrial scale equipment), most inactivation reactions are strongly accelerated (Toepfl, et al., 2006). Other benefits of this technology include the inactivation of some enzymes and gelation of proteins. It may also maintain nutritional and sensory attributes of food systems, and enable the development of products with novel characteristics (Barbosa-Cánovas et al., 2005).

In chapter 4 it was described the optimization of a mango bar product with an edible coating based on gellan gum. In the present chapter, the developed bars were packaged and their shelf life was evaluated focusing the work in two main topics:

(i) the study of the effect of processing treatments (TT and HHP) on mango bars microbial stability and release of volatile flavour compounds.

(ii) evaluation of bars properties during storage at refrigerated conditions (5 °C) during 21 days, after being subjected to TT. In particular, the colour (total colour difference and browning index), microbial contamination (aerobic mesophillic bacteria, yeasts and mould and *Listeria monocytogenes* bacteria), texture (Texture Profile Analysis evaluation of hardness, springiness and cohesiveness) and sensory quality were monitored over time.

5.3. Materials and methods

5.3.1. Materials

Gellan gum and mango fruits were acquired as described in section 2.3.1. – Chapter 2.

Similar volatile compounds were used as described in section 4.3.1. – Chapter 4.

5.3.2. Preparation of the samples

Preparation of the mango bars was performed as described in section 3.3.1. – Chapter 3.

The coating formulation used for this study (1.0 %wt L/H90/10 with the addition of 6 mM Ca^{2+}) was optimized in section 4.4.2. (Results and discussion) – Chapter 4. The preparation of the selected coating solution and application procedure to the mango bars are described in section 4.3.3. – Chapter 4.

5.3.3. Mango bars packaging

The obtained mango bars were packaged at atmospheric pressure in a multi-layered composite PET/PE aluminium film with the dimensions - 12 cm length x 8 cm width, which was heat sealed (Figure 5.1). The composite films combines the advantages of various layers of materials, polyethylene terephthalate (PET) and polyethylene (PE), providing a low oxygen transmission rate and a barrier for moisture loss and light (Haji-Saeid, Sampa, & Chmielewski, 2007).

Figure 5.1 Packaged coated mango bar.

5.3.4. Effect of thermal treatment and high hydrostatic pressure processing on microbial contamination and volatiles release of packaged mango bars

Thermal treatment (TT)

Thermal treatment was carried out in a water bath with temperature control. The packaged mango bars were immersed into the water bath and subjected to temperature/time conditions according to Table 5.2. Immediately after the treatment, the samples were cooled in chilled water for 5 min then stored at 5° C for 48 h before analysis. Microbial and volatile compounds analyses were carried out in duplicate for both control and treated samples at each temperature-time condition.

High hydrostatic pressure (HHP)

The samples were transported under refrigerated conditions to the high hydrostatic pressure equipment (QFP 350L-600 Avure Technologies Incorporated, Kent WA, USA), located in Alcobaça, Portugal. The packaged mango bars were placed in the cylindrical loading container (alloy steel vessel) at 6 °C and pressurized at 500 MPa during time periods: 120 and 240 seconds (Table 5.1). The treated samples were stored at 5 °C for 48 h before analysis.

Table 5.1.

Experimental conditions used in the thermal treatments and in high hydrostatic pressure essays

Process	Conditions
Thermal treatment	Temperature/time
TT1	80 °C / 120 s
TT2	80 °C / 300 s
TT3	90 °C / 120 s
TT4	90 °C / 300 s
High hydrostatic pressure at 6 °C	Pressure/time
HHP1	500 MPa / 120 s
HHP2	500 MPa / 240 s

Microbial analysis

The aerobic mesophillic microorganisms and yeasts/mould counts were made according to ISO 4833, (2003) and the Portuguese Standard 3277-1, (1978). A mass of 10 g of each sample and 90 mL of Tryptone salt (Biokar diagnostics) were placed in a stomacher® bag and homogenized using a Stomacher 400 (IUL Instruments, Barcelona, Spain). Further decimal dissolutions were made with the same diluent, and duplicates of at least three appropriate dilutions were placed on appropriate media. To enumerate the aerobic mesophillic microorganisms, 1.0 mL of each dilution was pour-plated in Plate Count Agar (PCA, Biokar diagnostics). After incubation at 30 °C for 72 h, the plates with 15-300 colonies were counted. To count yeasts and mould, 0.1 mL of each subsequent dilution was spread on a Dichloran Rose Bengal Chloramphenicol agar (SRBC, Oxoid, England) plate and incubated at 30 °C for 5 days.

Volatiles release

Samples preparation was performed as described in section 4.3.7. – Chapter 4. A mass of 5 g from the refrigerated sample (5 °C) was transferred into a 40 mL glass vial (suitable for headspace gas chromatography analysis).

The analysis of the volatile compounds was performed by Headspace-Solid Phase Micro Extraction-Gas Chromatography (HS-SPME-GC) as described in section 4.3.7. – Chapter 4. Two replicates were carried out for each sample.

5.3.5. Quality evaluation of in-package thermal treated (TT) mango bars during storage at 5 °C

Thermal treatment of in-package mango bars was carried out as described in section 5.3.4 at one single temperature/time condition (80 °C/120 s), then stored at 5 °C for 21 days and analysed on days 0, 4, 7, 17 and 21 of storage. Non treated samples were prepared, stored and analysed on same days. Analytical control includes:

Colour measurements

The colour of the mango bars was determined with a colorimeter (Minolta CR 300, Switzerland). CIELAB, L* (lightness), a* (red-green) and b* (yellow-blue) colour space was applied to perform colour measurements and the total colour difference (ΔE*) were performed as described in section 4.3.6. – Chapter 4. Here, the value ΔE* represents the colour difference between day 0 and each day of analysis.

Browning index (BI) was calculated according to the equation 5.1 (Hirschler, 2012):

$$BI = [100(x \times 0.31)]/0.172 \quad \text{Eq. 5.1}$$

where, x is calculated as follows:

$$x = (a^* + 1.75L^*)/(5.645L^* + a^* - 3.012b^*).$$

Texture Profile Analysis

Texture Profile Analysis was carried out as described in section 3.3.4. – Chapter 3.

Microbial load

The efficiency of in-package thermal treatment was monitored by microbial growth over 21 days of storage at 5 °C. Determination of aerobic mesophillic microorganisms and yeasts/mould was performed as described in section 5.3.4.

Detection of *Listeria monocytogenes* pathogen was performed following the procedure ISO 11290-1/A1, (2005). A mass of 25 g of sample was diluted in 225 mL Fraser Half Concentration broth (Biokar diagnostics), homogenized and incubated at 37 °C for 48 h. Portions of 0.2 mL of culture from the enriched Fraser broth were surface plated on each of three replicate plates of PALCAM agar (Merck, Darmstadt, Germany) and incubated aerobically at 37 °C for 48 h.

Sensory evaluation

Sensory evaluation of the mango bars was carried out in a standardized test room (see section 3.3.6 – Chapter 3). The mango bars were previously removed from the package and attributes such as - colour, flavour, taste, texture, off-flavour and overall acceptance were analysed by ten panellists at regular time intervals. The ten panellists (8 females and 2 males), aged between 25 and 55 years old,

were students or staff members of Instituto Superior de Agronomia (Lisbon, Portugal). Having in mind the attributes of a fresh mango fruit, the panellists were asked to compare the colour, flavour and taste attributes of the samples relatively to those of a fresh mango fruit following the 9 scale from 1 = not similar to 9 = very similar. Texture and off-flavour were evaluated and scored in a scale from 1 to 9 where 1 = extremely undesirable and 9 = extremely desirable. Control and treated mango bars were served in a plate, coded with three-digit numbers and randomly provided to the panellists (ISO 8587:2006). Control (untreated) and treated samples were analysed only until the 7^{th} and 17^{th} days of storage, respectively, due to safety reasons.

5.3.6. Statistical analysis

One-way variance analysis (ANOVA) using the software STATISTICA version 6.0 (2001) (StatSoft Inc., Tulsa, OK, USA) was performed to evaluate the statistical significant differences between estimated parameters of the samples. Hedonic ratings of the mango bars quality were used to evaluate the consumer preference on sensory attributes and purchasing decision.

5.4. Results and discussion

5.4.1. Effects of TT and HHP on microbial contamination and volatiles release of packaged mango bars

Microbial contamination

Microbial load results of the mango bars subjected to TT and HHP processing are shown in Table 5.2. No significant differences were detected in the contamination with aerobic mesophillic bacteria and with yeasts/moulds after the application of the different thermal treatments. The exception was the TT of 90°C / 300 s, that reduced the microbiological counts of aerobic mesophillic bacteria from 2.5 $\log_{10}$ CFU g^{-1} (control) to less than 1 $\log_{10}$ CFU g^{-1} (samples treated). Yeast and moulds counts were less than 2 $\log_{10}$ CFU g^{-1} for all the conditions applied.

Table 5.2

Effect of thermal treatment (TT) and high hydrostatic pressure (HHP) on the microbial load of the mango bars

Treatment	Conditions	Aerobic mesophillic bacteria[a] Log CFU g^{-1}	Yeasts/moulds Log CFU g^{-1}
TT	Control	2.5 ± 0.21[a]	< 2
	80°C / 120 s	1.12 ± 0.59[a]	< 2
	80°C / 300 s	1.77 ± 0.32[a]	< 2
	90°C / 120 s	2.05 ± 0.17[a]	< 2
	90°C / 300 s	< 1	< 2
HHP	Control	< 1	< 2
	500 MPa / 120 s	< 1	< 2
	500 MPa / 240 s	< 1	< 2

[a] Values reported as mean ± standard deviation (n = 2)

Aerobic mesophillic counts for untreated (control) and pressurized samples were 2.5 and less than 1 $\log_{10}$ CFU g^{-1}, respectively. Yeasts/moulds counts were less than 2 $\log_{10}$ CFU g^{-1} for both treatments (Table 5.3). Guerrero-Beltran et al., (2006) have also reported that pressurized mango puree at 483 MPa and 552 MPa for 1, 3 and 5 min also had no significant microbial contamination.

It can be noticed that the microbial load is quite low even for the untreated samples. In the course of mango bars preparation, mango puree was heated at 88 ±2 °C for 30 seconds which probably lowered the microbial activity of the produced mango bars before being packaged and subjected to both TT and HPP treatments. In 2012, the European Commission of Health and Consumer Protection, highlighted that the limit for aerobic mesophillic bacteria and yeast/moulds counts is < 10^5 CFU g^{-1} and < 10^3 CFU g^{-1} respectively. The results indicate that the control bars and the ones subjected to both high pressure and thermal treatments, under the conditions tested, present a quite low microbial contamination.

Volatiles release

The aim of this study was to evaluate the impact of high hydrostatic pressure and thermal treatments on the release of aroma volatile compounds. Figure 5.2 shows the peak areas of the 8 studied volatile compounds released by heat treated (Figure 5.2a) pressurized mango bars (Figure 5.2b), and their corresponding control samples. It should be emphasized that, according to the methodology used

to analyse the aroma compounds release, the higher the peak area, the lower amount of volatiles was released during the TT and HPP processes (section 4.4.7 – Chapter 4). The peaks presented in Figure 5.2 correspond to the amount of volatiles still present in the bars after the treatments.

Figure 5.2a shows that for all conditions, δ-3-carene has a significant major peak area when compared with the other volatile compounds. Furthermore, no substantial differences were observed in the peak areas of δ-3-carene between control and treated mango bars, either by TT of HHP. This compound was described as possessing a mango-like and a typical aroma of mango leaves and was found to be present in high concentrations in various mango cultivars (Table 4.1 – Chapter 4) (Malundo, Baldwin, Moshonas, Baker, & Shewfelt, 1997). Moreover, in section 4.4.3 – Chapter 4, in which was studied the effect of coating on mango bars on volatile release; δ-3-carene presented a similar predominance over the whole volatile compounds analysed. For ϒ-decalactone, geraniol and nerol, no substantial differences were also encountered between treated samples and control by different TT and HHP conditions.

Regarding menthone and Y-octalactone higher peak area are observed for mango bars treated at 90 °C for 120 s. For HPP treated bars, menthone peak areas show a tendency to decrease with increasing pressure time, while in the case of Y-octalactone the opposite tendency is observed. For terpinolene, only the bars treated at 90 °C for 120 s present a substantial lower peak area. Regarding decanal, lower peak areas are observed after all heat treatments, however, no significant differences are seen between the two HPP treatments and the control sample.

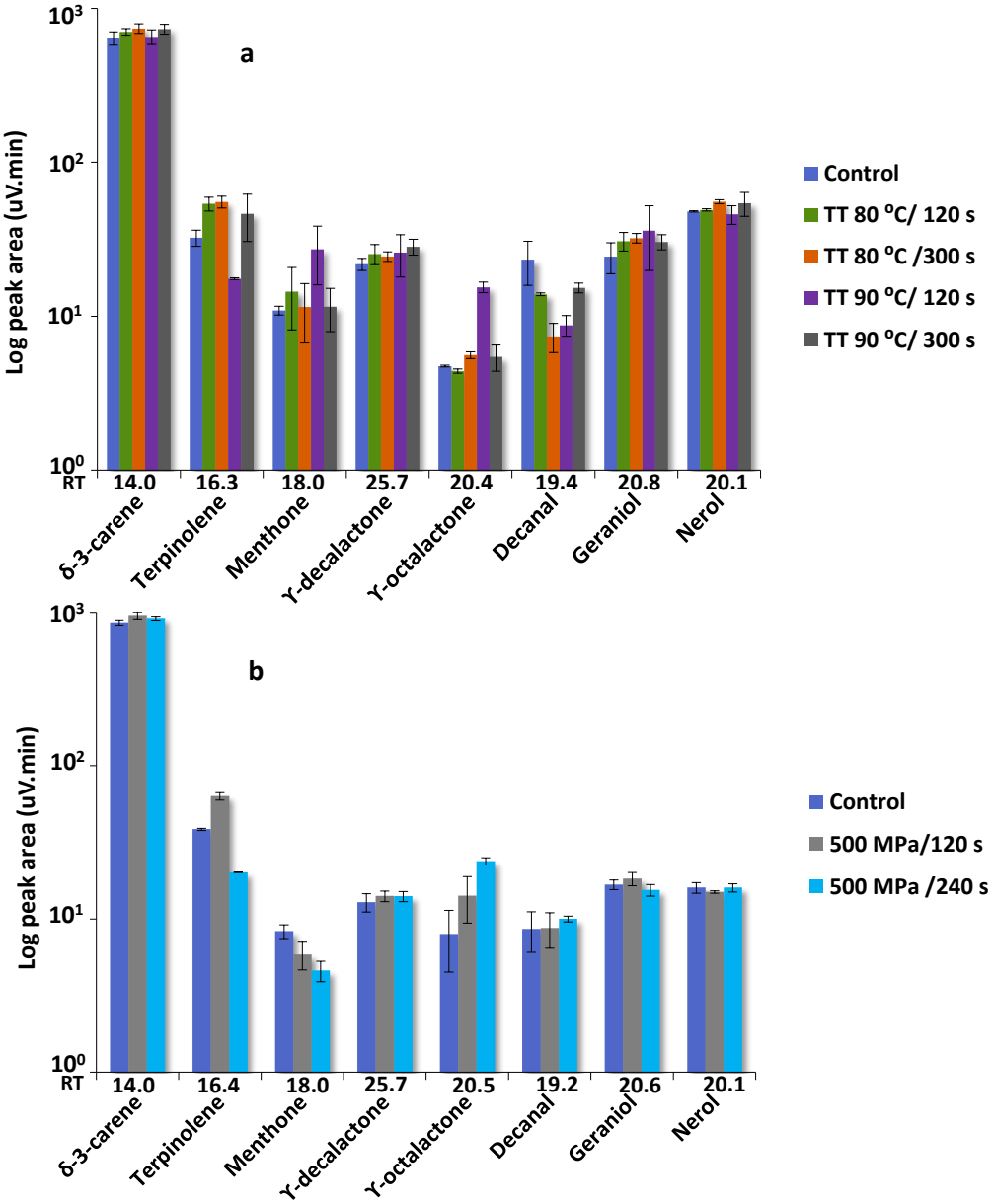

Figure 5.2 Mean values (± standard deviation) and retention times (min) (RT) of volatile compounds of coated mango bars treated by: (a) thermal treatment (TT) and (b) high hydrostatic pressure treatment (HHP).

It should be noticed that, except for δ-3-carene, the value of the peak areas obtained was low, indicating that the volatiles analytical method may be improved, and doing so, a more supported analysis may be performed. Still, the results above suggest that TT and HHP do not affect significantly the aroma profiles of the mango bars. It may be related to the fact that, according to the mango bars production process, mango puree is thermally pre-treated at 88 ± 2 °C for 30 s in order to dissolve gellan gum and to deactivate enzymes (see section 3.3.1, chapter 4). The aroma compounds with

higher volatility could have been already released during that operation. Thus, when applying the TT and HPP treatments to mango bars, no substantial effect on the aroma profile is noticed.

Both conservation treatments applied can be used to achieve an effective microbiological control. So, treatments with the milder conditions might be the best option to suppress future microbiological growth during storage. With respect to the obtained results, and the increased equipment requirements for HHP, it was selected the in-package thermal treatment at 80 °C for 120 s as the treatment for mango bars to assure a satisfactory shelf-life.

5.4.2. Properties of the heat treated mango bars during storage

Colour

Fruit products are attractive and eye-catching because of the richness of pigments that they contain. Preservation of yellow and orange carotenoids in mango bars is of vital importance to maintain the quality. Non-enzymatic browning of the mango bars could result upon heating or storage after processing the bars.

Colour alterations of the samples during storage time are shown in Figure 5.3. ΔE* values for control and treated mango bars range from 1 to 4. On day 4 of storage, total colour difference for control increased until 2.2, while treated mango bars reached a value of 3.7 (Figure 5.3a). Control and treated samples maintained their constant values of colour difference until day 17, followed by a slight decrease.

The browning index (BI) represents the purity of brown colour and is an important parameter in processes where enzymatic or non-enzymatic browning takes place (Kaushik, Kaur, Rao, & Mishra, 2014). No significant differences were noticed between the browning index (BI) of both control and treated samples, BI ranged between 98 and 102 for the control and 98 to 104 for treated bars, over the storage time.

Overall, the colour was stable during refrigerated storage for untreated and treated mango samples. These results are very important reflecting the stability of natural pigments present in mango fruit such as carotenoids. Most likely, pre-heating and thermal treatments caused inactivation of enzymes responsible for the degradation of carotenoids (Santhirasegaram, Razali, George, & Somasundram, 2014).

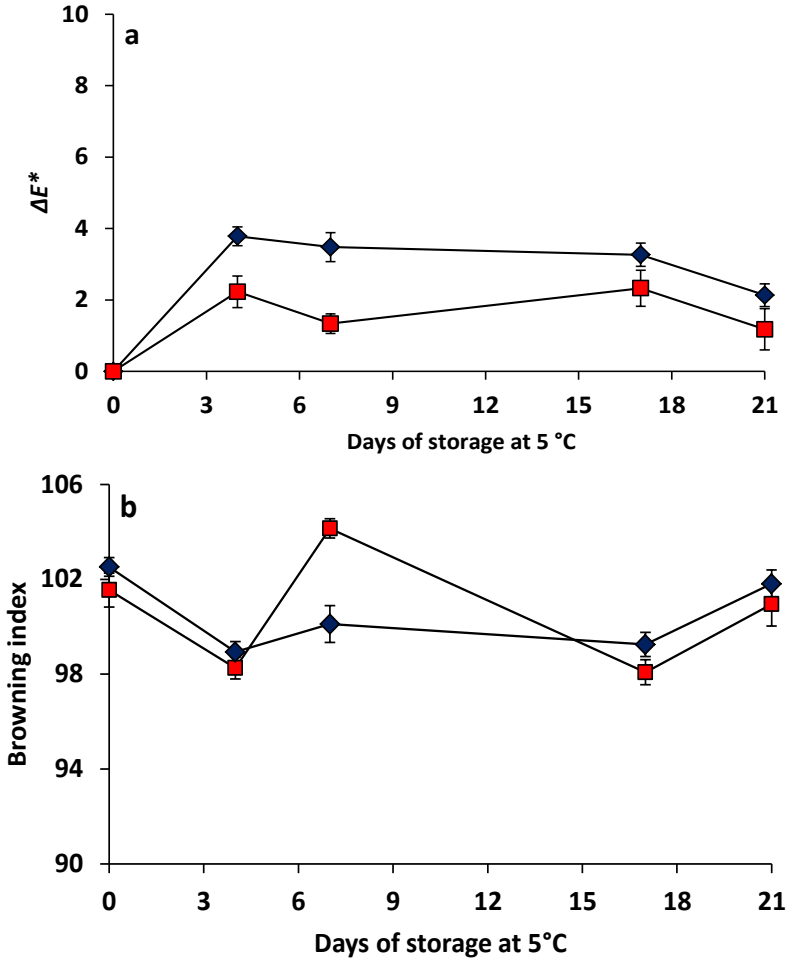

Figure 5.3 Effect of thermal treatment on the total colour difference (ΔE*) and browning index (BI) of mango bars during storage at 5 ^{0}C. Symbols: Diamond - control; squares - thermal treatments at 80 °C for 120 s. Bars indicate mean ± standard deviation.

TPA analysis

Appearance of a fruit product plays an important role on consumer's evaluation and they take appearance into account upon their purchases of the fruit products. As mentioned earlier, colour has a great impact on appearance but quality loss is also observed throughout changes in texture, which is another important quality criteria.

The texture properties of heat treated (80 °C for 120 s) and untreated mango bars during storage at 5 °C for 21 days, were evaluated by TPA (Figure 5.4). The values of hardness, springiness and cohesiveness showed statistically non-significant differences ($p > 0.05$) between the treated and untreated samples over the entire storage time. Moreover, the referred properties were practically constant during the storage period, except for hardness, that tended to decrease in the last day. The results indicate that all the mango bars maintained their texture during the whole storage period.

The stability observed in both control and heat treated mango bars may be related to the thermal pre-treatment at 88 ± 2 °C for 30 s of the mango puree upon bars production. This treatment may have

provided suitable conditions for enzymes deactivation such as polygalacturonase (PG), which is responsible for the textural softening of food products (Singh, & Dwivedi, 2008). Houben et al., (2013) verified that high temperatures (70 ºC) for a relatively long time (600 s), almost entirely deactivated polygalacturonase PG. Being this enzyme sensitive to high temperature thermal treatments, probably the exposure at 88 ± 2 °C for 30 seconds was sufficient to inactivate PG in the mango puree.

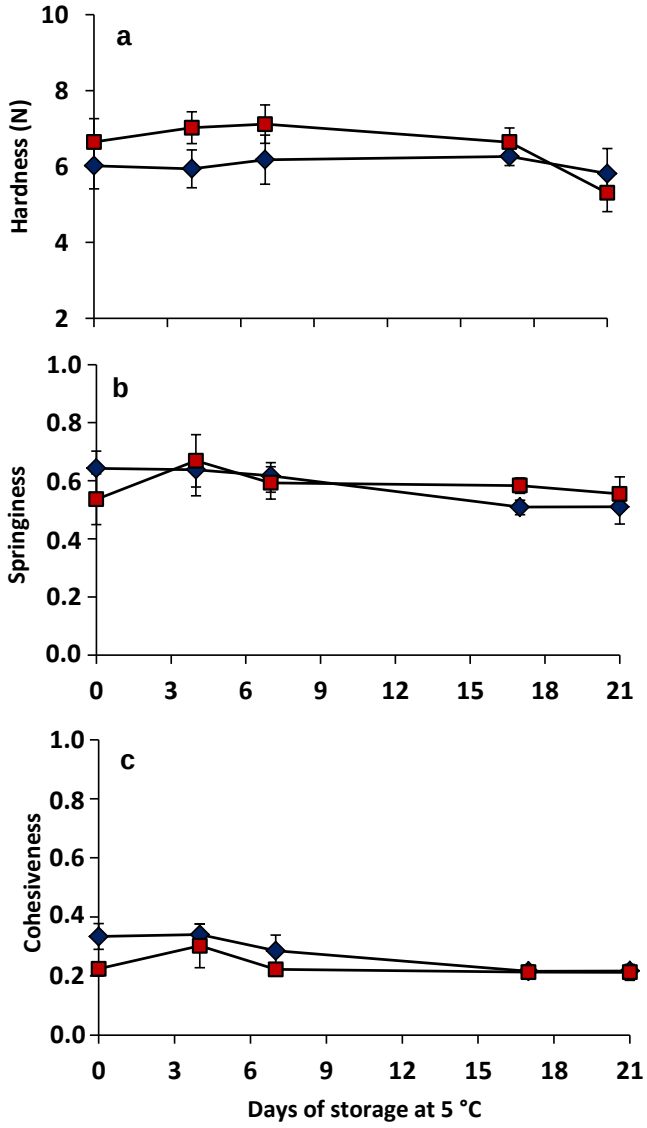

Figure 5.4 TPA parameters of mango bars over 21 days of refrigerated storage, symbols: diamond - control; squares - thermal treatment at 80°C for 120 s. Bars indicates mean ± standard deviation.

Microbial load

The counts of aerobic mesophillic bacteria (AMB) and yeast and moulds (Y&M) for control and heat treated mango bars during storage are shown in Figure 5.5.

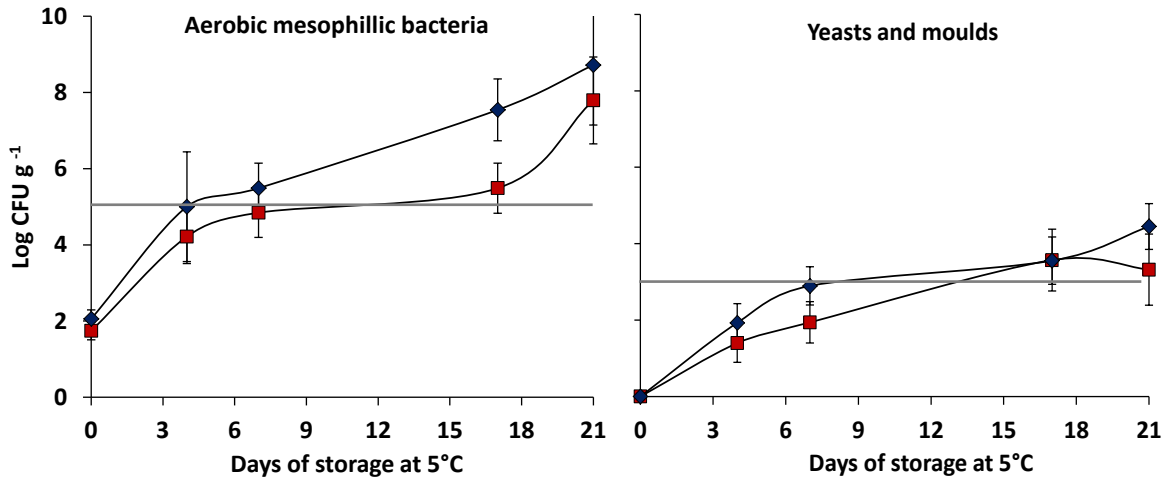

Figure 5.5 Effect of thermal treatment on aerobic mesophillic bacteria, moulds and yeasts of the mango bars during refrigerated storage. Symbols: diamond - control; squares - mango bars treated at 80 °C for 120 s. Bars indicate mean ± standard deviation. Grey horizontal line represents the limit according to the legislation (European Commission of Health and Consumer Protection, 2012).

The low initial AMB contamination of treated samples is attributed to the thermal treatments, either of the mango puree during mango bars production, or of the in-packaged mango bars before storage. The AMB counts increased during storage time for both samples. However, it reached the limit at day 7 for the untreated sample, while for treated samples this limit was achieved only after 17 days. It is reported in the literature that TT caused a significant reduction in aerobic mesophillic bacteria of mango juice treated at 90 °C for 60 s (Santhirasegaram et al., 2014). Also, high temperature short time treatment (HTST) at 110 °C/8.6 s reduced total aerobic bacteria in purple sweet potato nectar (Wang et al., 2012). In this work, the stability during storage may be attributed to the very low initial AMB contamination along with the low pH (< 5.0) of mango bars, which may have suppressed the growth of the surviving bacteria.

The counts of Y&M in treated samples were maintained below 3.0 log CFU g^{-1} during the 21 days of refrigerated storage. However, for untreated samples the limit was reached after 17 days. Zhao et al., (2013) also referred that thermal treatment (85˚C/15 s) of cucumber juice drink reduced significantly the Y&M growth was reduced significantly.

Listeriosis infection is associated with the consumption of minimally processed and ready-to-eat foods contaminated with *Listeria monocytogenes* bacteria during post-processing step such as slicing,

pealing and packaging (Aureli, et al., 2000; Murphy et al., 2005). *L. monocytogenes* bacteria was absent in both non-treated and treated mango bars during refrigerated storage.

Sensory analysis

Results from the sensory analysis of the mango bars during refrigerated storage are presented in a spider web diagrams for control and heat treated mango bars (Figure 5.6). Through sensory analysis, no significant difference was identified between the considered attributes of treated/untreated samples. The panellists did not identify differences in colour of control and treated samples, presenting high scores (8) during whole storage period (Figure 5.6a). This is in line with the results presented in the colour measurements with low total colour change (ΔE*) values indicating that colour alterations were not significant. Flavour and taste attributes present high scores for both treated and untreated mango bars. Off-flavour, such as cooking flavour was not detected for all the samples. In-package thermal treatment at 80 °C for 120 seconds did not affect the flavour of mango bars. Regarding the texture, treated/untreated mango bars scored lower values than the other attributes, but still with a good texture acceptance by the panellists. The overall acceptance level was satisfactory, as 76 % and 78 % were favourable towards control and treated mango bars respectively.

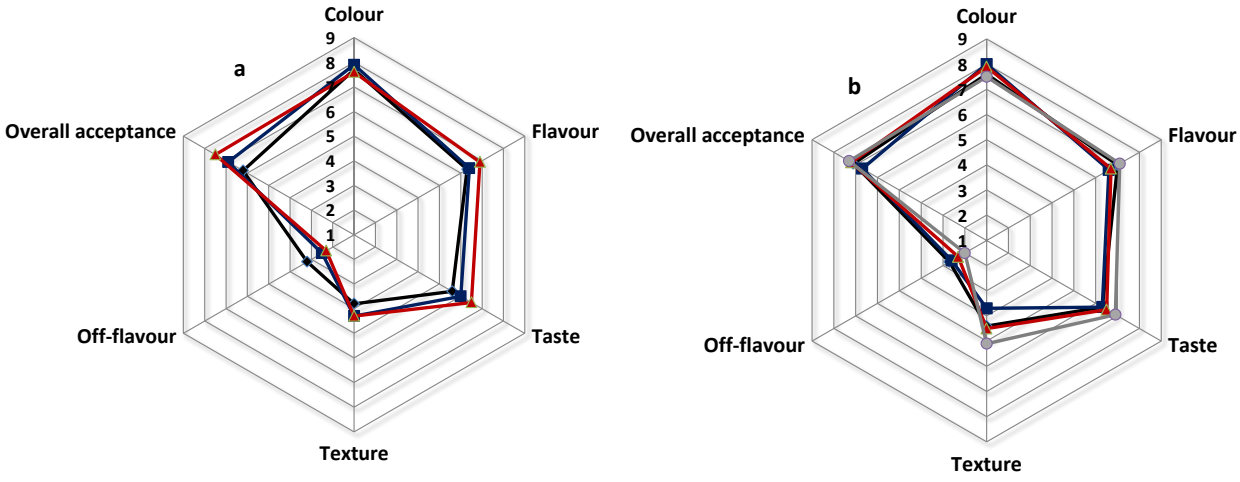

Figure 5.7 Spider diagrams for mango bars sensory attributes during storage at 5 °C, (a) control (b) mango bars treated at 80 °C for 120 s. Simbols: (♦) – day 0, (■) – day 4, (▲) – day 7, (●) – day 17.

Due to the soft texture of the mango bars, the packaging was performed at atmospheric pressure in order to avoid mechanical injuries with the applied vacuum. Multi-layered composite aluminium package provided an oxygen, water and light barrier.

The shelf life of the developed product is stablished only by the microbial safety, as the sensory properties did not change either with a mild TT (80°C/120 s) or with storage time under refrigerated conditions.

Multivariate analysis of mango bars quality parameters

To study the influence of different factors on mango bars quality, a multivariate method (Principal Component Analysis) was performed. The two principal components (PC1 and PC2) accounted for 75.29 % of the total variability observed (Figure 5.7). Variables with higher scores on PC1 are related with hardness and colour (ΔE* and BI) parameters. PC2 corresponded to variables related to microbial parameters.

The first division of all the samples is made into two main clusters, at a Euclidian distance of 3. The variables of treated mango bars, grouped on the positive side on day 0 and 7 and control on day 0, explained 52.64 % of the total variance with hardness, total colour difference (ΔE*) and browning colour (BI). The highest contribution on PC2 corresponded to variables related to microbial parameters of treated and untreated mango bars, grouped on the positive side, indicating that the storage time had a greater impact on microbial load than the thermal treatment.

The untreated mango bars on day 4 (C4) was more distant from the other groups having a low contribution to the discriminating groups. The total colour difference was negatively correlated with hardness and browning index, helping to find out which variables contributed the most to the difference among the groups.

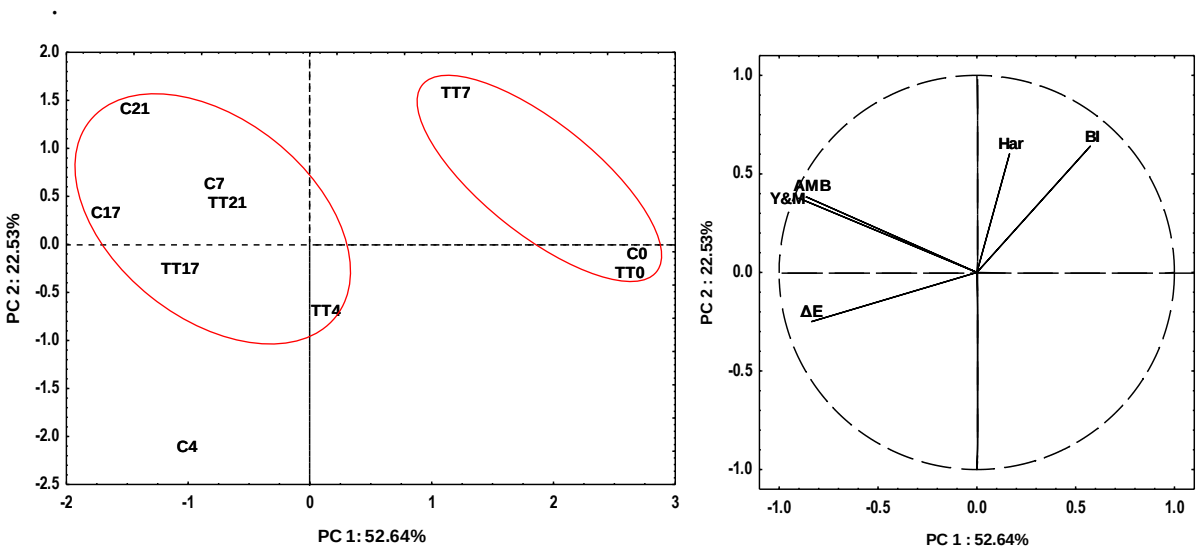

Figure 5.7 The loading plots of the hardness; colour and microbial load of thermal treated (TT) and control (C) mango bars during 21 days of refrigeration. 0, 4, 7, 17, 21 are the days of analysis. ΔE* – total colour difference; BI – browning index; Har – Hardness; Y&M – yeast and moulds, AMB – aerobic mesophillic bacteria.

5.5. Conclusions

Ready-to-eat mango bars, were subjected to in-package thermal treatment (80 and 90 °C for 120 and 300 s) and high hydrostatic pressure treatment (500 MPa for 120 and 240 seconds), in order to compare their performance. Analysis of the impact on flavour release and microbial stability of the mango bars showed that both treatments do not affect significantly the aroma profiles of the mango bars and that both are effective to achieve a very low microbial contamination.

Based on these results, heat treatment at 80ºC for 120 s was selected as the preservation technique and its impact on the properties of the mango bars during refrigerated storage (5 ºC) for 21 days was studied. The results of hardness, springiness and cohesiveness, obtained by TPA analysis, showed non-significant differences ($p > 0.05$) between the treated and untreated samples. All the mango bars maintained their firm texture during storage. The colour was also stable during refrigerated storage for all the samples.

Sensory analysis showed that thermal treatment did not have any detrimental effect on sensory quality of mango bars as no significant difference was identified between the considered attributes of treated/untreated samples.

Microbiologically the product has shown to be quite stable. It is envisaged that the thermal treatment for enzymatic inactivation and dissolution of gellan gum applied to the mango puree during bars production had an important effect. *Listeria monocytogenes* bacteria was absent in both untreated and treated samples.

The shelf life of the developed product is established only by the microbial safety, as the sensory properties did not change either with a mild TT (80 ˚C/120 s) or with storage time under refrigerated conditions: 17 days for TT samples and 7 days for untreated samples.

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Chapter 6

General Conclusions and Future Research

6.1. General conclusions

The work described in this thesis, was directed to the development of a value-added novel ready-to-eat mango fruit based product. Another important aspect is the use of already mature fruits that in a short period of time would suffer a drastic decrease in their overall quality, making them unsuitable for consumption as fresh fruit. The fruit chosen in this work was mature mango due to its flavour, and significant amounts of bioactive compounds with antioxidant activity and also because the casual consume of this fruit is not easy.

The novel ready-to-eat product developed was a convenient mango bar, based on mango puree and gellan gum as gelling agent. The chemical properties of the mango puree used, namely the content of cations and water, pH and TSS were compatible with the hydration and gelation conditions of gellan gum. The addition of either low (L) or high (H) acyl gellan gum or L/H ratios to the mango puree produced mango bars with interesting viscoelastic properties. Microscopic observations (Confocal Laser Scanning Microscopy and Cryo-scanning Electron Microscopy) highlighted the formation of a denser structure with lower pore size for mango bars obtained with only L acyl gellan and a more loosened structure with larger pores and thin strings for bars with only H acyl gellan.

Formulations based on mango puree with a gellan overall concentration of 1.0 %wt, varying the low acyl/high acyl gellan ratio (L/H) enabled, to produce mango bars with diverse physical properties, which were grouped in two different texture profiles:

(i) bars with higher hardness and brittleness, lower syneresis by passive diffusion, corresponding to L/H ratios of 75/25, 50/50 and only L gellan

(ii) bars with softer and more cohesive structure with a good elastic recovery, which presented a higher syneresis by passive diffusion and, corresponding to those with a L/H ratio of 25/75 and with only H gellan.

Sensory evaluation allowed to identify the preferred mango bar formulation in terms of texture which was the one prepared with a L/H ratio of 25/75. The proportion of L gellan was enough to impart the suitable firmness to the product, while H gellan was responsible for the softness of the mango bar.

Considering that an edible coating can act as an additional barrier that may improve the appearance retention of the volatile compounds, and minimize syneresis, the development of a compatible coating based on gellan gum was performed. It was concluded that 1.0 %wt of L/H in the ratio of 90/10 with addition of 6 mM calcium gluconolactate could represent the optimal coating formulation for the mango bars.

The effect of different preservation treatments (thermal treatment and high hydrostatic pressure) applied on packaged mango bars on the microbial counts and aroma release showed that both treatments do not significantly affect the aroma profiles of the mango bars and that both are effective

to achieve microbial stability. Based on these results, a heat treatment at 80ºC for 120 s was selected as the preservation technique.

In order to evaluate the product shelf life, thermal treated packaged mango bars were stored under refrigerated conditions (5 ºC) for 21 days. It was observed that during that period the product was stable in terms of texture, colour and microbial growth. Furthermore, the bars had also a good sensory acceptance by the sensory panel during all the conservation period.

From a microbiologically point of view, the stability, can also be attributed to the thermal treatment of mango puree for enzymatic inactivation that takes place during bars production. *Listeria monocytogenes* bacteria was absent in all packaged mango bars, subjected or not to thermal treatment at 80ºC for 120 s after packaging.

The results obtained are very encouraging, demonstrating that there is a synergism between mango puree and gellan gum being able to produce a stable ready-to-eat mango bar appreciated by the sensory panellists. The processing costs are reasonable; taking into account that gellan gums have a relatively low cost because of its simplicity of manufacturing. Mango fruit cannot be considered a cheap tropical fruit, however ripe mangoes that will became over ripen in a short period of time can be the main ingredient for mango bars production. In view of this, and also of its ease of consumption, ready-to-eat mango bars could have an enormous potential to be included in the group of processed food product with health promoting benefits.

The shelf life of the developed product, 17 days, was stablished only by the microbial safety, as the sensory properties did not change either with a mild TT (80˚C/120 s) or with storage time under refrigerated conditions.

6.2. Future research

This thesis presents an approach for the development of a healthy and convenient mango fruit product – texturized mango bar. Still, the production process proposed may be further enhanced.

Only one mango cultivar was selected, however, the results suggest the viability of the project and the interest in extending it to other mango cultivars. In fact the fruit chemical composition may vary with the type of cultivar, and consequently, changes of the properties of the mango bars could occur. It will also be interesting to extend this work to other fruits, particularly those with a relevant profile of bio-active compounds and which consumption is not easy as fresh fruit.

Pectins are prominent structural constituents in the mango fruit and together with gellan gum can contribute to the final texture of the mango bars. Due to complex chemical composition of mango pulp, special studies need to be done in the future in order to understand the impact of pectin present in the processed mango puree, taking into account the complex molecular interactions in multicomponent food systems such as mango pulp.

Volatile compounds from mango bars should be analysed by combining gas chromatography with mass spectroscopy in order to better proceed to their identification, and their quantification should also be performed.

Even though the results of conservation studies of packaged bars were promising in terms of maintenance of the overall quality of the product (e.g. in terms of texture, water holding capacity, microbial growth, etc), it will be important to extend the shelf-life of the ready-to-eat mango bars, for example developing a bioactive coating composed by hydrocolloids and essential oils or using a modified atmosphere packaging.

Future analysis can also be done in order to understand the stability of mango bars at lower values of pH in order to evaluate other applications of this product, as for example its introduction in yogurt formulations.

It is also important to understand and investigate the steps required to scale-up the production process, and take this product to a commercial level, including cost/benefit analysis and the search for the best route to exploitation, for example by collaborative research with industrial partners.

Appendix

Appendix A – Time sweeps of mango puree with gellan gum

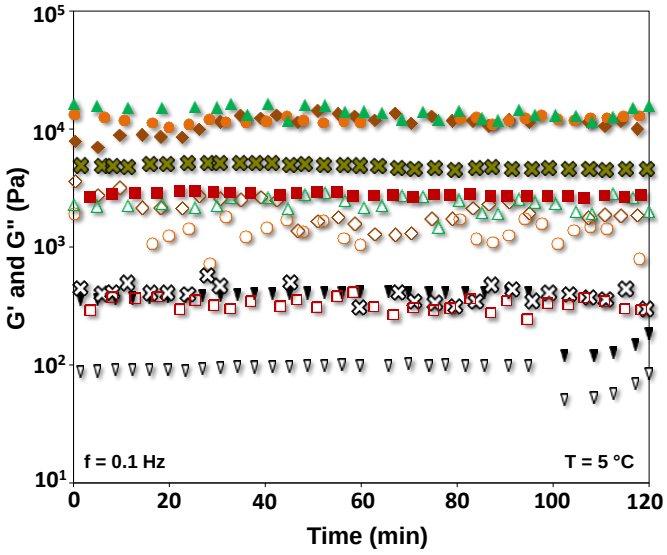


Figure A.1 Time sweeps of mango puree with L, H and different L/H gellan ratios for a fixed overall gellan concentration (1 %wt); full symbols – storage modulus (G'), open symbols– loss modulus (G"); (▼) control, (♦) L, (●) 75/25, (▲) 50/50, (✖) 25/75, (■) H.

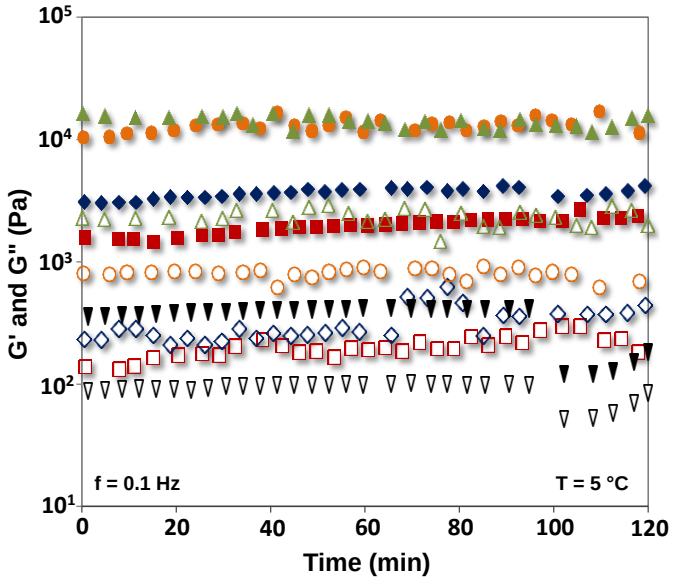
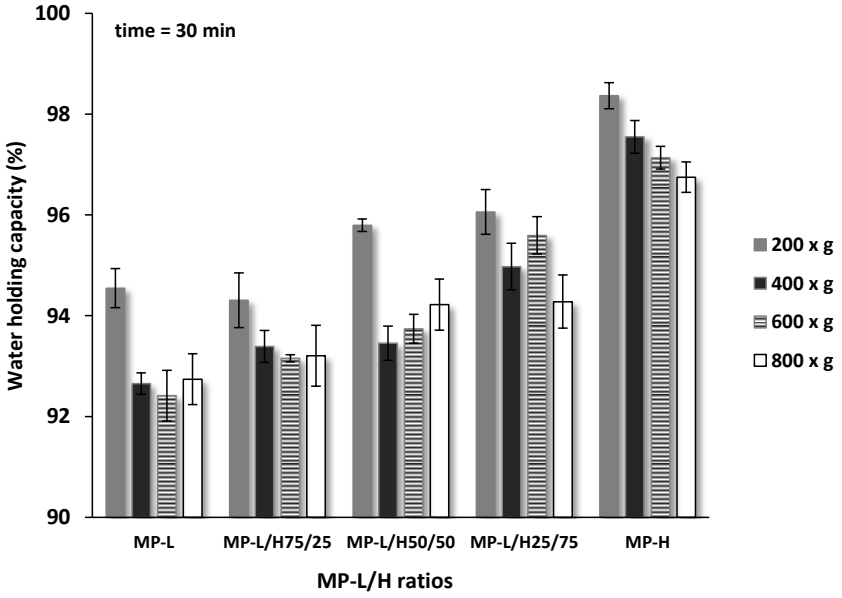


Figure A.2 Time sweeps of mango puree with L/H 50/50 at different gellan concentrations; full symbols – storage modulus (G'), open symbols– loss modulus (G"); (▼) control, (■) 0.25 %, (♦) 0.5 %, (▲) 1.0 %, (●) 2.0 %

Appendix B- Influence of centrifugation force on water holding capacity of mango bars at different L/H ratios

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan)



Appendix C- Stress-relaxation curves (SR) of mango puree and at different ratios of L/H gellan gum. Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan)

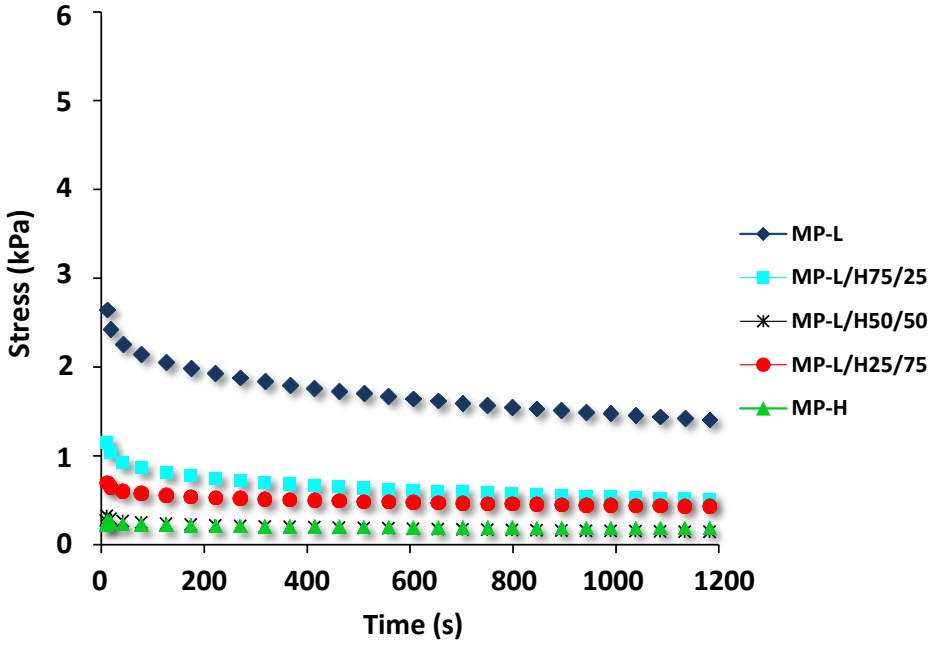


Figure C.1 SR curves of MP-L/H samples for 5 % strain and a cross-head speed of 0.05 mm s^{-1}

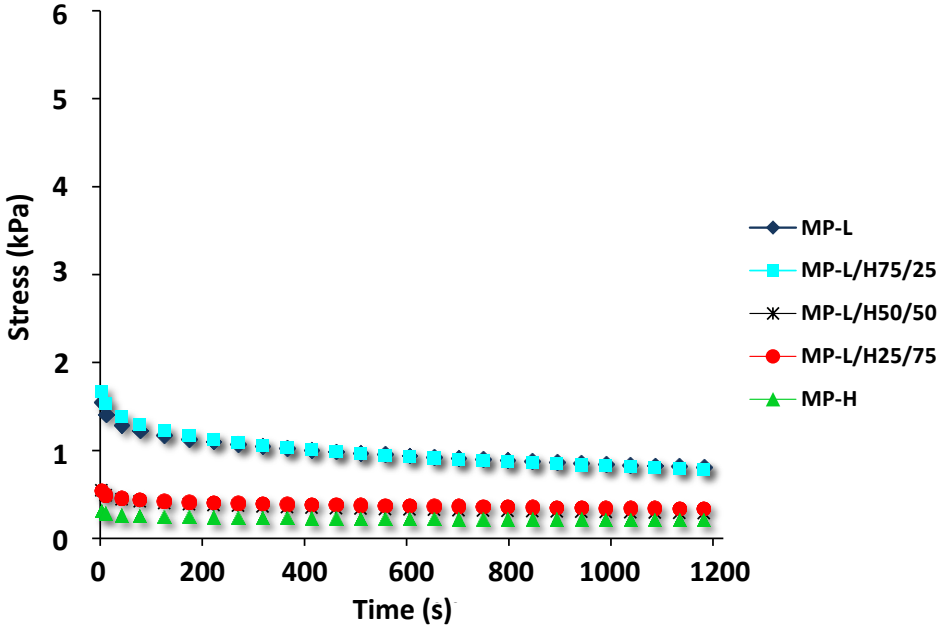


Figure C.2 SR curves of MP-L/H samples for 5 % strain and a cross-head speed of 0.5 mm s^{-1}

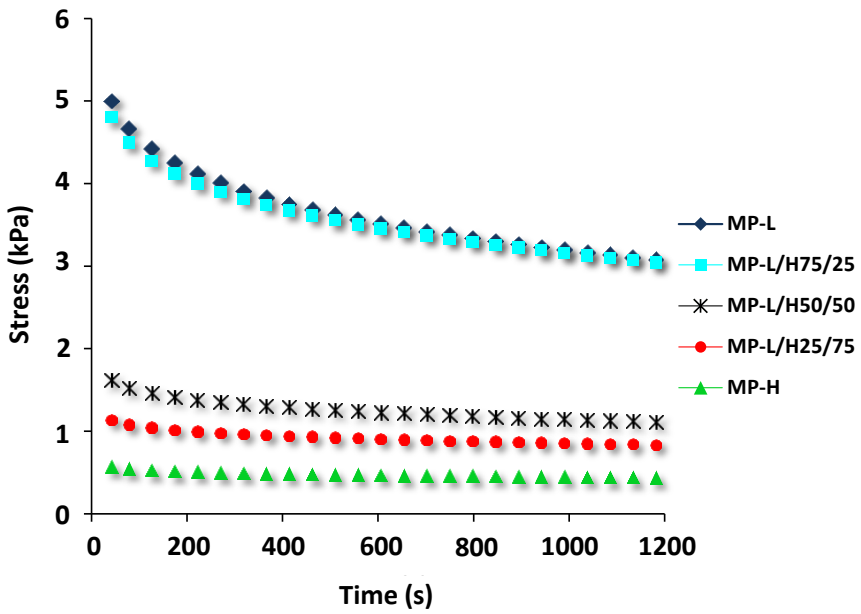
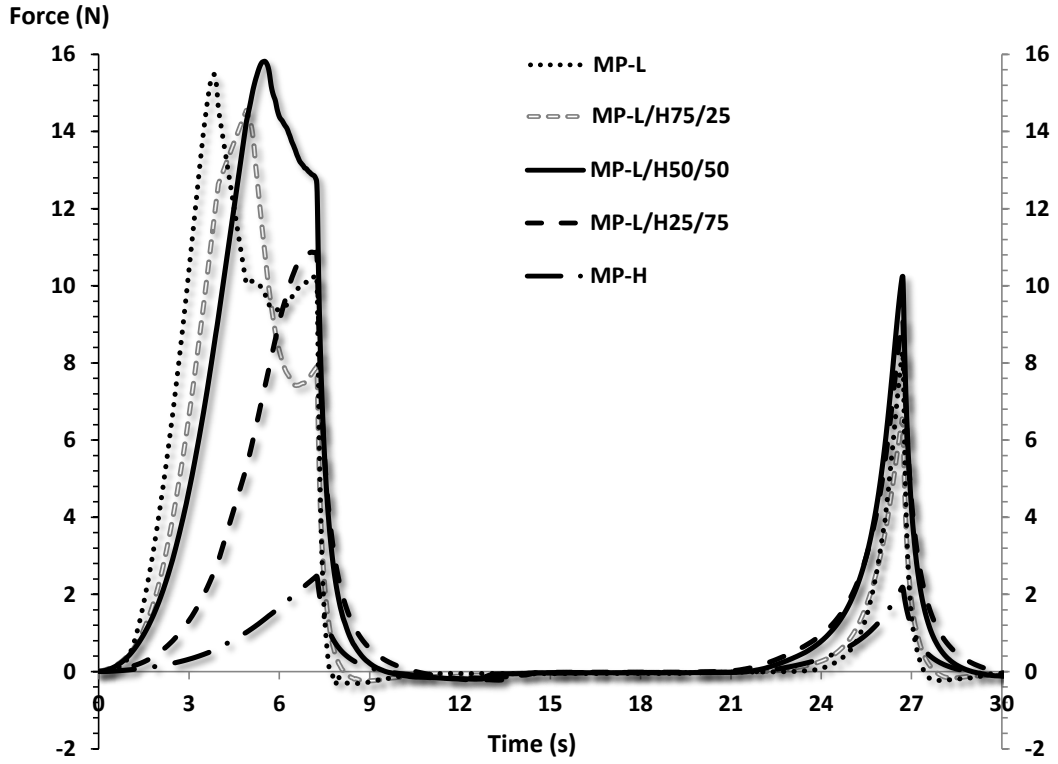


Figure C.3 SR curves of MP-L/H samples for 10 % strain and a cross-head speed of 0.05 mm s^{-1}

Appendix D - Force vs. Time curves of MP with different L/H gellan ratios.

Legend: MP-L (mango puree with only low acyl gellan), MP-L/H75/25 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-L/H50/50 (mango puree with a low acyl/high acyl gellan ratio of 50/50), MP-L/H25/75 (mango puree with a low acyl/high acyl gellan ratio of 75/25), MP-H (mango puree with only high acyl gellan).



Appendix E – Chromatograms of the mango bars

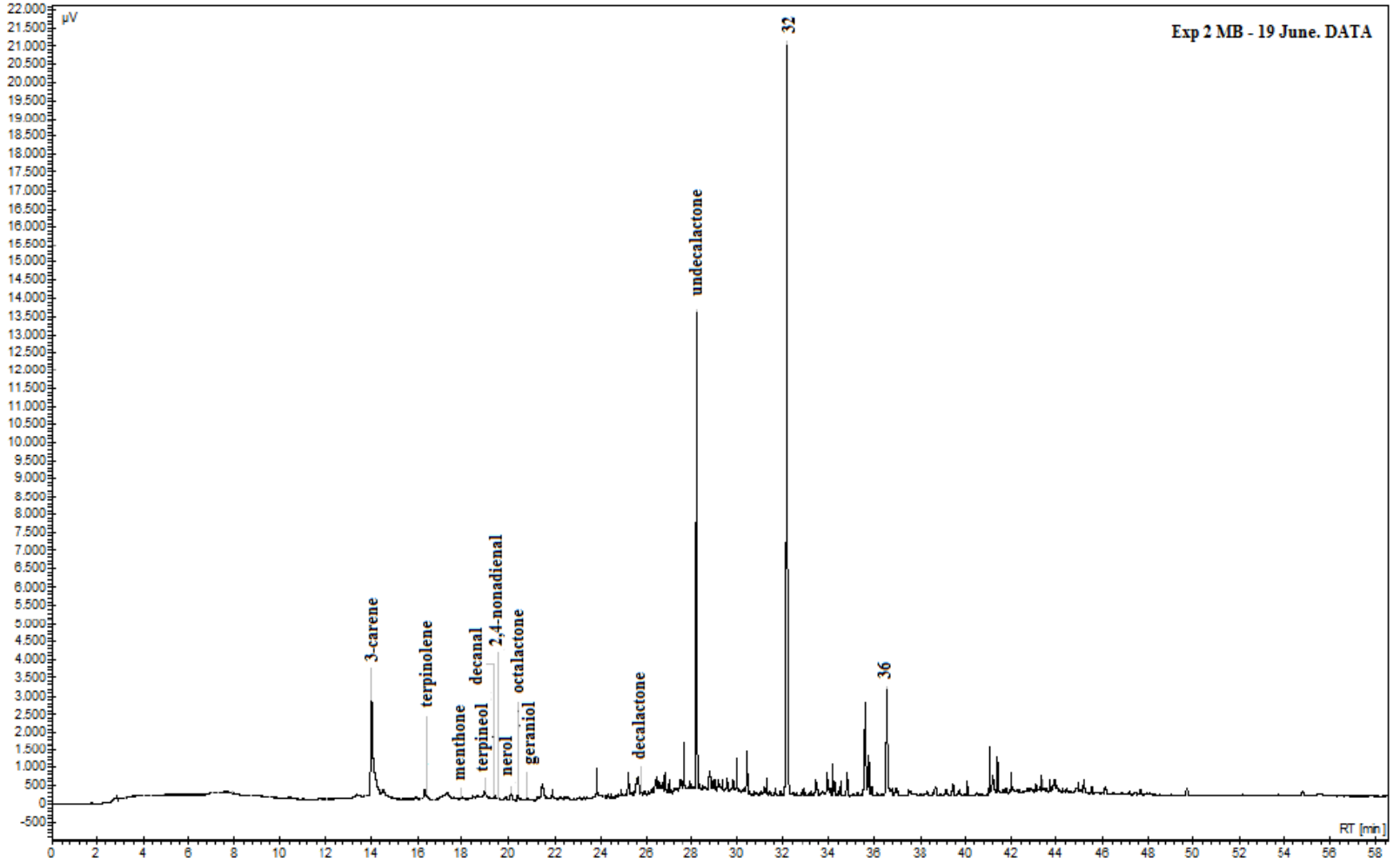


Figure E.1 Chromatogram obtained from mango puree and 1 %wt L/H 25/75 (glass vial) of the selected volatiles

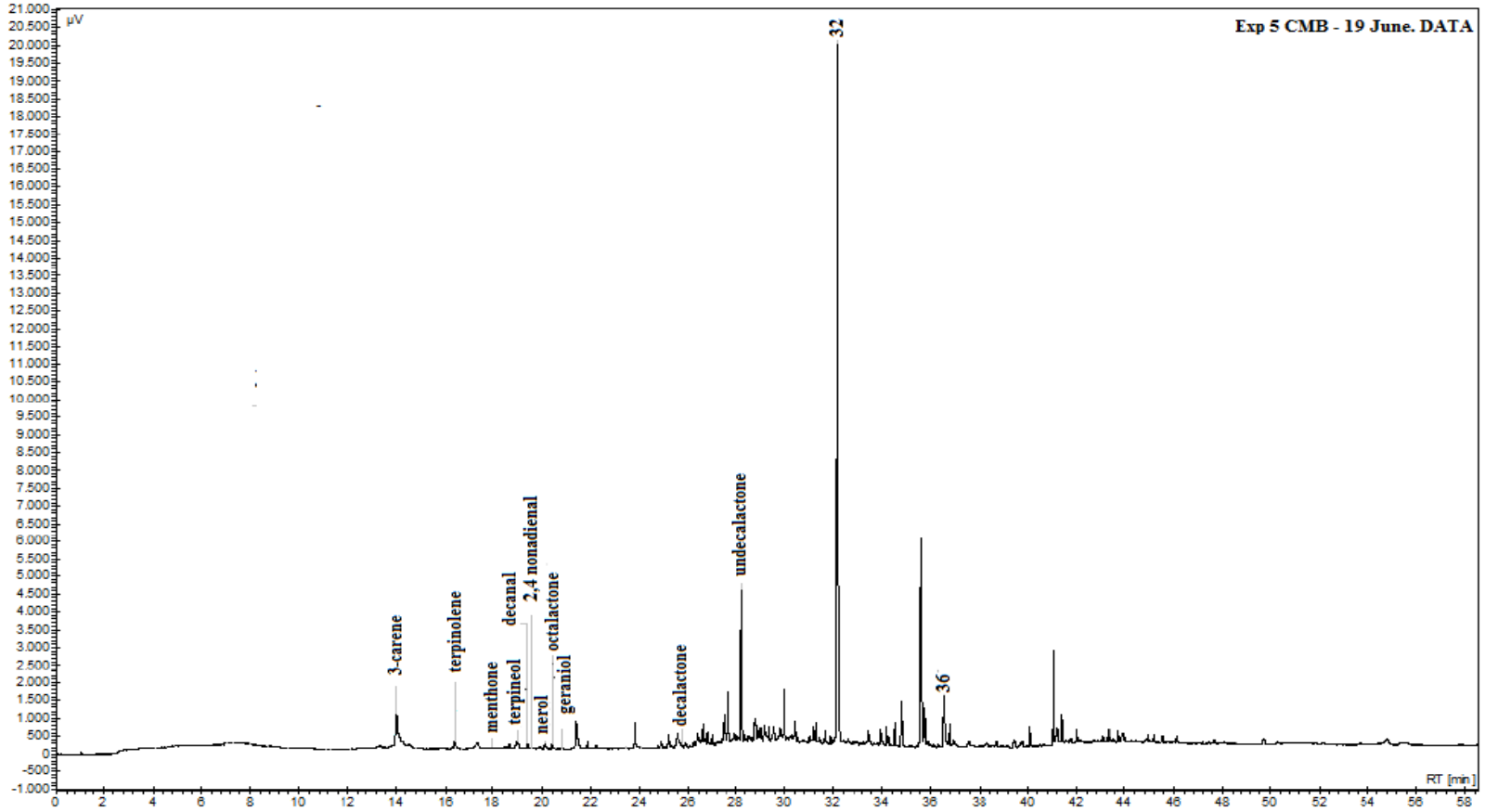


Figure E.2 Chromatogram obtained from mango puree and 1 %wt L/H 25/75 and coating based on gellan gum L/H 90/10 and 6 mM Ca^{2+} (glass vial)